IMPROVED HEAT SHOCK PROTEIN-BASED VACCINES AND IMMUNOTHERAPIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. § 119(e) to provisional applications serial no. 60/462,469, filed April 11, 2003; serial no. 60/463,746, filed April 18, 2003; and serial no. 60/503,417, filed September 16, 2003, all three of which are incorporated herein by reference in their entireties.

INTRODUCTION

[0002] The present invention relates to methods and compositions for inducing an immune response in a subject, wherein the subject is administered an effective amount of at least one or more defined hybrid antigens optionally in combination with one or more heat shock proteins. These methods and compositions may be used in the treatment of infectious diseases and cancers.

BACKGROUND OF THE INVENTION

[0003] Heat shock proteins were originally observed to be expressed in increased amounts in mammalian cells which were exposed to sudden elevations of temperature, while the expression of most cellular proteins is significantly reduced. It has since been determined that such proteins are produced in response to various types of stress, including glucose deprivation. As used herein, the term "heat shock protein" will be used to encompass both proteins that are expressly labeled as such as well as other stress proteins, including homologues of such proteins that are expressed constitutively (i.e., in the absence of stressful conditions). Examples of heat shock proteins include BiP (also referred to as grp78), hsp70, hsc70, gp96 (grp94), hsp60, hsp40 and hsp90.

[0004] Heat shock proteins have the ability to bind other proteins in their non-native states, and in particular to bind nascent peptides emerging from ribosomes or extruded into the endoplasmic reticulum. Hendrick and Hartl, *Ann. Rev. Biochem.* 62:349-384 (1993); Hartl, *Nature* 381:571-580 (1996). Further, heat shock proteins have been shown to play an

important role in the proper folding and assembly of proteins in the cytosol, endoplasmic reticulum and mitochondria; in view of this function, they are referred to as "molecular chaperones." Frydman et al., *Nature* 370:111-117 (1994); Hendrick and Hartl, *Ann. Rev. Biochem.* 62:349-384 (1993); Hartl, *Nature* 381:571-580 (1996).

[0005] For example, the protein BiP, a member of a class of heat shock proteins referred to as the hsp70 family, has been found to bind to newly synthesized, unfolded μ immunoglobulin heavy chain prior to its assembly with light chain in the endoplasmic reticulum. Hendershot et al., *J. Cell Biol.* 104:761-767 (1987). Another heat shock protein, gp96, is a member of the hsp90 family of stress proteins which localizes in the endoplasmic reticulum. Li and Srivastava, *EMBO J.* 12:3143-3151 (1993); Mazzarella and Green, *J. Biol. Chem.* 262:8875-8883 (1987). It has been proposed that gp96 may assist in the assembly of multi-subunit proteins in the endoplasmic reticulum. Wiech et al., *Nature* 358:169-170 (1992).

[0006] It has been observed that heat shock proteins prepared from tumors in experimental animals were able to induce immune responses in a tumor-specific manner; that is to say, heat shock protein purified from a particular tumor could induce an immune response in an experimental animal which would inhibit the growth of the same tumor, but not other tumors. Srivastava and Maki, *Curr. Topics Microbiol.* 167:109-123 (1991). Genes encoding heat shock proteins have not been found to exhibit tumor-specific DNA polymorphism. Srivastava and Udono, *Curr. Opin. Immunol.* 6:728-732 (1994). High resolution gel electrophoresis has indicated that gp96 may be heterogeneous at the molecular level. Feldweg and Srivastava, *Int. J. Cancer* 63: 310-314 (1995). Evidence suggests that the source of heterogeneity may be populations of small peptides adherent to the heat shock protein, which may number in the hundreds. *Id.* It has been proposed that a wide diversity of peptides adherent to tumor-synthesized heat shock proteins may render such proteins capable of eliciting an immune response in subjects having diverse HLA phenotypes, in contrast to more traditional immunogens which may be somewhat HLA-restricted in their efficacy. *Id.*

[0007] Nieland et al. (*Proc. Natl. Acad. Sci. U.S.A.* 93:6135-6139 (1996)) identified an antigenic peptide containing a cytotoxic T lymphocyte (CTL) vesicular stomatitis virus (VSV) epitope bound to gp96 produced by VSV-infected cells. Neiland's methods

precluded the identification of any additional peptides or other compounds which may also have bound to gp96, and were therefore unable to further characterize higher molecular weight material which was bound to gp96 and detected by high pressure liquid chromatography.

[0008] It has been reported that a synthetic peptide comprising multiple iterations of NANP (Asp Ala Asp Pro) malarial antigen, chemically cross-linked to glutaraldehyde-fixed mycobacterial hsp65 or hsp70, was capable of inducing antibody formation (i.e., a humoral response) in mice in the absence of any added adjuvant; a similar effect was observed using heat shock protein from the bacterium *Escherichia coli*. Del Guidice, *Experientia* 50:1061-1066 (1994); Barrios et al., *Clin. Exp. Immunol.* 98:224-228 (1994); Barrios et al., *Eur. J. Immunol.* 22:1365-1372 (1992). Cross-linking of synthetic peptide to heat shock protein and possibly glutaraldehyde fixation was required for antibody induction. Barrios et al., *Clin. Exp. Immunol.* 98:229-233.

[0009] PCT/US96/13363 describes hybrid antigens comprising an antigenic domain and a heat shock protein binding domain that, in a complex with a heat shock protein, induces immunological responses to antigens and are thus useful for treatment of cancer and infectious diseases. PCT/US98/22335 describes additional heat shock protein binding domains for similar uses, as well as the ability for hybrid antigen administered alone to induce an immune response. It has now been discovered that improvements in the peptide linker present between the at least one antigenic domain and at least one heat shock protein binding domain in a hybrid antigen leads to an increase in biological activity. This increase is also found to provide an increase in inducing an immune response against the antigenic portion of the hybrid antigen. It is towards these improved peptide linkers, hybrid peptides containing them and their uses with and without heat shock protein, that the present application is directed.

SUMMARY OF THE INVENTION

[0010] The present invention relates to methods and compositions for inducing an immune response in a subject, wherein at least one defined hybrid antigen optionally in a complex with a heat shock protein is administered to the subject. The hybrid antigen

comprises at least one antigenic domain and at least one heat shock protein binding domain, and at least one peptide linker there between. Induction of an immune response to an antigen associated with a disease such as an infectious disease or tumor is useful for treatment of the disease. The antigenic or immunogenic domain of the hybrid antigen may be an entire protein or peptide antigen, or may be only a portion of the selected antigen, for example a selected epitope of the antigen. The heat shock protein binding domain is a peptide that binds to a heat shock protein, preferably a peptide of 7-15 amino acids that binds to a heat shock protein, more preferably a hydrophobic peptide that binds to a heat shock protein, and most preferably a hydrophobic peptide of 7-15 amino acids that binds to a heat shock protein. The linker has a sequence from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred.

[0011] The present invention provides for methods of administering such hybrid antigens alone, as well as heat shock protein/hybrid antigen compositions, the latter comprising (i) combining one or more heat shock protein with one or more hybrid antigens *in vitro*, under conditions wherein binding of hybrid antigen to heat shock protein occurs to form a hybrid antigen/heat shock protein complex; and (ii) administering the hybrid antigen, bound to heat shock protein, in an effective amount to a subject in need of such treatment.

[0012] Alternatively, hybrid antigens optionally in combination with heat shock protein may be introduced into a subject by administering to the subject a nucleic acid encoding the hybrid antigen, optionally with nucleic acid encoding the heat shock protein.

[0013] Thus, in a first aspect, the invention is directed to a hybrid antigen consisting essentially of an antigenic domain of an infectious agent or tumor antigen, a binding domain

that non-covalently binds to a heat shock protein, and a peptide linker separating the antigenic domain and the binding domain, and wherein the peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred.

[0014] In a second aspect, the invention is directed to a hybrid antigen consisting essentially of a plurality of antigenic domains of one or more infectious agents or one or more tumor antigens, at least one binding domain that non-covalently binds to a heat shock protein, and at least one peptide linker separating the antigenic domains and the at least one binding domain, and wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred. In a particular embodiment, at least one of the antigenic domains in the aforementioned hybrid antigen is a T helper epitope.

[0015] In a third aspect, the invention is directed to a hybrid antigen comprising an antigenic domain of an infectious agent or tumor antigen and a binding domain that non-covalently binds to a heat shock protein, and a peptide linker there between, and wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe

Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred. In a particular embodiment, the aforementioned hybrid antigen has a peptide linker separating the antigenic domain and the binding domain.

[0016] In a fourth aspect, the invention is directed to a hybrid antigen comprising a plurality of antigenic domains of one or more infectious agents or one or more tumor antigens and at least one binding domain that non-covalently binds to a heat shock protein, and at least one peptide linker there between, and wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred. In a particular embodiment, at least one of the antigenic domains is a T helper epitope.

[0017] In a fifth aspect, the invention is directed to a composition for inducing an immune response to an infectious agent or tumor antigen comprising at least one hybrid antigen, the hybrid antigen comprising an antigenic domain of the infectious agent or tumor antigen, a binding domain that non-covalently binds to a heat shock protein, and at least one peptide linker there between, and wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID

NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred. In one embodiment, the composition comprises a plurality of hybrid antigens, and one of the hybrid antigens can comprise a T helper epitope.

[0018] In a sixth aspect, the invention is directed to a composition for inducing an immune response to an infectious agent or tumor antigen comprising at least one hybrid antigen, the hybrid antigen comprising a plurality of antigenic domains at least one of which is from the infectious agent or tumor antigen, at least one binding domain that non-covalently binds to a heat shock protein, and at least one peptide linker there between, and wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred.

[0019] In a seventh aspect, the invention is directed to a composition for inducing an immune response to an infectious agent or tumor antigen comprising at least one hybrid antigen, the hybrid antigen consisting essentially of an antigenic domain of the infectious agent or tumor antigen, a binding domain that non-covalently binds to a heat shock protein, and a peptide linker separating the antigenic domain and the binding domain, and wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln

Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred. In one embodiment, the aforementioned composition comprises a plurality of hybrid antigens. In another aspect, at least one of the plurality of hybrid antigens comprises a T helper epitope.

[0020] In an eighth aspect, the invention is directed to a composition for inducing an immune response to an infectious agent or tumor antigen comprising at least one hybrid antigen, the hybrid antigen consisting essentially of a plurality of antigenic domains at least one of which is from the infectious agent or tumor antigen, at least one binding domain that non-covalently binds to a heat shock protein, and at least one peptide linker separating the antigenic domain and the binding domain, and wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. In one embodiment, at least one of the antigenic domains comprises a T helper epitope.

[0021] In a ninth aspect, the invention is directed to a method for inducing an immune response to an infectious agent or tumor antigen comprising administering to a subject a complex of a heat shock protein and a hybrid antigen comprising at least one antigenic domain of the infectious agent or tumor antigen, at least one binding domain comprising a peptide that non-covalently binds to a heat shock protein, and a peptide linker there between; wherein the hybrid antigen and the heat shock protein are non-covalently bound, and wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys

Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred. In one embodiment, the complex comprises a plurality of hybrid antigens. In an embodiment, at least one of the hybrid antigens is a T helper epitope. In another embodiment, the hybrid antigen comprises a plurality of antigenic domains, and at least one of the antigenic domains can be a T helper epitope. In yet another embodiment wherein the complex comprises a plurality of hybrid antigens, at least one of the hybrid antigens comprises a plurality of antigenic domains. In another embodiment of this aspect of the invention, the heat shock protein is a hsp70.

[0022] In a tenth aspect, the invention is directed to a method for inducing an immune response to an infectious agent or tumor antigen comprising administering to a subject a complex of a heat shock protein and a hybrid antigen, the hybrid antigen consisting essentially of at least one antigenic domain of an infectious agent or tumor antigen, a binding domain that non-covalently binds to a heat shock protein, and a peptide linker separating the antigenic domain and the binding domain, and wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred. In one embodiment, the complex comprises a plurality of hybrid antigens. In another embodiment, at least one of the hybrid antigens is a T helper epitope. In a further embodiment, the hybrid antigen comprises a plurality of antigenic domains. In yet a further

embodiment, at least one of the antigenic domains is a T helper epitope. In still yet another embodiment, the complex comprises a plurality of hybrid antigens, at least one of the hybrid antigens comprising a plurality of antigenic domains. In a preferred embodiment of this aspect, the heat shock protein is a hsp70.

[0023] In an eleventh aspect, the invention is directed to a method for inducing an immune response to an infectious agent or tumor antigen comprising administering to a subject at least one hybrid antigen comprising at least one antigenic domain of the infectious agent or tumor antigen, at least one binding domain comprising a peptide that non-covalently binds to a heat shock protein, and at least one peptide linker there between, and wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred. In one embodiment, the complex comprises a plurality of hybrid antigens. In another embodiment, at least one of the hybrid antigens is a T helper epitope. In another embodiment, the hybrid antigen comprises a plurality of antigenic domains. In a further embodiment, at least one of the antigenic domains is a T helper epitope. In yet a further embodiment, the complex comprises a plurality of hybrid antigens, at least one of the hybrid antigens comprising a plurality of antigenic domains. In another embodiment of this aspect of the invention, a peptide linker separates the antigenic domain and the binding domain.

[0024] In a twelfth embodiment, the invention is directed to a method for inducing an immune response to an infectious agent or tumor antigen comprising administering to a subject at least one hybrid antigen, the hybrid antigen consisting essentially of at least one antigenic domain of an infectious agent or tumor antigen, a binding domain that non-covalently binds to a heat shock protein, and a peptide linker separating the antigenic

domain and the binding domain, and wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred. In one embodiment, the complex comprises a plurality of hybrid antigens. In a further embodiment, at least one of the hybrid antigens is a T helper epitope. In another embodiment, the hybrid antigen comprises a plurality of antigenic domains. In yet another embodiment, at least one of the antigenic domains is a T helper epitope. In yet still a further embodiment, the complex comprises a plurality of hybrid antigens, at least one of the hybrid antigens comprising a plurality of antigenic domains.

[0025] In a thirteenth aspect, the invention is directed to a method for treating an infectious disease or cancer comprising administering to a subject a complex of a heat shock protein and a hybrid antigen comprising at least one antigenic domain of an infectious agent or tumor antigen associated with the infectious disease or cancer, a binding domain comprising a peptide that non-covalently binds to a heat shock protein, and a peptide linker there between; and wherein the hybrid antigen and the heat shock protein are non-covalently bound, and wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred. In one embodiment, the complex

comprises a plurality of hybrid antigens. In another embodiment, at least one of the hybrid antigens is a T helper epitope. In yet another embodiment, the hybrid antigen comprises a plurality of antigenic domains. In still another embodiment, at least one of the antigenic domains is a T helper epitope. In yet still a further embodiment, the complex comprises a plurality of hybrid antigens, at least one of the hybrid antigens comprising a plurality of antigenic domains. In an embodiment of this aspect of the invention, a peptide linker separates the antigenic domain and the binding domain. In a preferred embodiment of this aspect of the invention, the heat shock protein is a hsp70.

[0026] In a fourteenth aspect, the invention is directed to a method for treating an infectious disease or cancer comprising administering to a subject a complex of a heat shock protein and a hybrid antigen, the hybrid antigen consisting essentially of at least one antigenic domain of an infectious agent or tumor antigen associated with the infectious disease or cancer, at least one binding domain that non-covalently binds to a heat shock protein, and a peptide linker separating the antigenic domain and the binding domain, and wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred. In one embodiment, the complex comprises a plurality of hybrid antigens. In another aspect, at least one of the hybrid antigens is a T helper epitope. In yet another aspect, the hybrid antigen comprises a plurality of antigenic domains. In yet another aspect, at least one of the antigenic domains is a T helper epitope. In a further aspect, the complex comprises a plurality of hybrid antigens, at least one of the hybrid antigens comprising a plurality of antigenic domains. In a preferred embodiment, the heat shock protein is a hsp70.

[0027] In a fifteen aspect, the invention is directed to a method for treating an infectious disease or cancer comprising administering to a subject at least one hybrid antigen comprising at least one antigenic domain of an infectious agent or tumor antigen associated with the infectious disease or cancer, a binding domain comprising a peptide that non-covalently binds to a heat shock protein, and a peptide linker there between, and wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred. In one embodiment, the complex comprises a plurality of hybrid antigens. In another aspect, at least one of the hybrid antigens is a T helper epitope. In yet another aspect, the hybrid antigen comprises a plurality of antigenic domains. In still a further aspect, at least one of the antigenic domains is a T helper epitope. In still yet another aspect, the complex comprises a plurality of hybrid antigens, at least one of the hybrid antigens comprising a plurality of antigenic domains. In one embodiment of this aspect of the invention, a peptide linker separates the antigenic domain and the binding domain.

[0028] In a sixteenth aspect, the invention is directed to a method for treating an infectious disease or cancer comprising administering to a subject at least one hybrid antigen, the hybrid antigen consisting essentially of at least one antigenic domain of an infectious agent or tumor antigen associated with an infectious disease or cancer, a binding domain that non-covalently binds to a heat shock protein, and a peptide linker separating the antigenic domain and the binding domain, and wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine,

wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred. In one embodiment, the complex comprises a plurality of hybrid antigens. In another embodiment, at least one of the hybrid antigens is a T helper epitope. In yet another embodiment, the hybrid antigen comprises a plurality of antigenic domains. In still yet another embodiment, at least one of the antigenic domains is a T helper epitope. In another embodiment, the complex comprises a plurality of hybrid antigens, at least one of the hybrid antigens comprising a plurality of antigenic domains.

[0029] In a seventeenth aspect, the invention is directed to a peptide from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V.

[0030] In an eighteenth aspect, the invention is directed to an immunogenic polypeptide comprising a plurality of antigenic domains, at least one heat shock protein binding domain and at leatst one peptide linker there between wherein at least one peptide linker is from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred.

[0031] In a nineteenth aspect, the invention is directed to a polynucleotide encoding any of the hybrid antigens in the aforementioned first, second, third or fourth aspect.

[0032] In a twentieth aspect, the invention is directed to a method of inducing an immune response to an infectious disease or cancer comprising administering to a subject a polynucleotide encoding a hybrid antigen comprising an antigenic domain of an infectious agent or tumor antigen associated with the infectious disease or cancer, a heat shock protein binding domain, and a peptide linker there between from among Phe Phe Arg Lys (FFRK; SEQ ID NO:1000); Phe Arg Lys (FRK); Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002); Arg Lys Asn (RKN); Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003); Phe Arg (FR), Gln Leu Lys (QLK), Gln Leu Glu (QLE), Ala Lys Val Leu (AKVL; SEQ ID NO:1001); Lys Asn (KN); Arg Lys (RK); or AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V. Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred.

[0033] In a twenty-first aspect, the invention is directed a method of inducing an immune response to an infectious disease or cancer comprising administering to a subject a polynucleotide encoding a hybrid antigen as mentioned above, and a polynucleotide encoding a heat shock protein. In a preferred embodiment, the encoded heat shock protein is a hsp70.

[0034] In any or all of the aforementioned aspects of the invention, the infectious disease antigen may be derived from an infectious agent such as a bacterium, virus, protozoan, mycoplasma, fungus, yeast, parasite, or prion, by way of non-limiting example. A cancer or tumor antigen associated with cancer may be derived from a sarcoma, a lymphoma, a leukemia, or a carcinoma, a melanoma, carcinoma of the breast, carcinoma of the prostate, ovarian carcinoma, carcinoma of the cervix, colon carcinoma, carcinoma of the lung, glioblastoma, or astrocytoma, by way of non-limiting examples. The antigenic domain of an infectious agent or cancer comprises an antigen derived from or associated with the infectious disease or tumor antigen, such that induction of an immune response to the

antigen of the infectious agent or cancer antigen, respectively, is useful for treating the corresponding infectious disease or cancer.

[0035] This application claims priority under 35 U.S.C. § 119(e) to provisional applications serial no. 60/462,469, filed April 11, 2003; serial no. 60/463,746, filed April 18, 2003; and serial no. 60/503,417, filed September 16, 2003, all three of which are incorporated herein by reference in their entireties.

BRIEF DESCRIPTION OF THE DRAWING

[0036] Figure 1 shows the results of a tumor challenge study in which immunization using a hybrid antigen or complex of a hybrid antigen with a heat shock protein was performed, followed seven days later by challenge with a tumor expressing the antigen.

DETAILED DESCRIPTION OF THE INVENTION

[0037] For purposes of clarity of description, and not by way of limitation, the detailed description is divided into the following subsections:

(i) hybrid antigens,

(ii) heat shock proteins; and

(iii) methods of administration.

Hybrid Antigens

[0038] A hybrid antigen, according to the invention comprises at least one antigenic (immunogenic) domain, at least one heat shock protein-binding domain, and a peptide linker between at least two of these domains, wherein the peptide linker is among

Phe Phe Arg Lys (FFRK; SEQ ID NO:1000),

Phe Arg Lys (FRK);

Phe Arg Lys Asn (FRKN, SEQ ID NO: 1002);

Arg Lys Asn (RKN);

Phe Phe Arg Lys Asn (FFRKN, SEQ ID NO:1003);

Phe Arg (FR),
Gln Leu Lys (QLK),
Gln Leu Glu (QLE),
Ala Lys Val Leu (AKVL; SEQ ID NO:1001),
Lys Asn (KN);
Arg Lys (RK); or
AA_1-AA_2-AA_3-leucine, wherein AA_1 is A, S, V, E, G, L, or K, preferably V, more preferably S, and most preferably A ; AA_2 is K, V, or E, preferably E, more preferably V and most preferably K; and AA_3 is V, S, F, K, A, E, or T, preferably F, more preferably S and most preferably V.

[0039] Among the foregoing, Gln Leu Lys (QLK), Arg Lys (RK) and Ala Lys Val Leu (AKVL; SEQ ID NO:1001) are preferred, and Phe Phe Arg Lys (FFRK; SEQ ID NO:1000) is most preferred.

[0040] Thus, the hybrid antigen serves at least two functions, namely (i) it contains an epitope capable of inducing the desired immune response; and (ii) it is capable of physically binding to a heat shock protein. As will be noted below, such binding may occur in vivo such that administration of the hybrid antigen alone will induce the desired immune response and provide the desired therapeutic effect.

[0041] The term "antigen" as used herein, refers to a compound which may be composed of amino acids, carbohydrates, nucleic acids or lipids individually or in any combination.

[0042] The term "hybrid antigen," as used herein, refers to a compound which binds to one or more heat shock proteins and which is representative of the immunogen toward which an immune response is desirably directed. For example, where the immunogen is an influenza virus, the hybrid antigen may comprise a peptide fragment of the matrix protein of the influenza virus. As used herein, the term "immunogen" is applied to the neoplastic cell, infected cell, pathogen, or component thereof, towards which an immune response is to be elicited, whereas the hybrid antigen comprises a portion of that immunogen which can provoke the desired response and which binds to one or more heat shock proteins. In particular, the antigenic domain of the hybrid antigen is selected to elicit an immune

response to a particular disease or pathogen, including peptides obtained from MHC molecules, mutated DNA gene products, and direct DNA products such as those obtained from tumor cells.

[0043] While the invention may be applied to any type of immunogen, immunogens of particular interest are those associated with, derived from, or predicted to be associated with a neoplastic disease, including but not limited to a sarcoma, a lymphoma, a leukemia, or a carcinoma, and in particular, with melanoma, carcinoma of the breast, carcinoma of the prostate, ovarian carcinoma, carcinoma of the cervix, colon carcinoma, carcinoma of the lung, glioblastoma, astrocytoma, etc. Selections of melanoma antigens useful in hybrid antigens of the present invention may be found, by way of non-limiting example, in PCT/US01/12449 (WO0178655), incorporated herein by reference in its entirety. Further, mutations of tumor suppressor gene products such as p53, or oncogene products such as ras may also provide hybrid antigens to be used according to the invention.

[0044] In further embodiments, the immunogen may be associated with an infectious disease, and, as such, may be a bacterium, virus, protozoan, mycoplasma, fungus, yeast, parasite, or prion. For example, but not by way of limitation, the immunogen may be a human papilloma virus (see below), a herpes virus such as herpes simplex or herpes zoster, a retrovirus such as human immunodeficiency virus 1 or 2, a hepatitis virus, an influenza virus, a rhinovirus, respiratory syncytial virus, cytomegalovirus, adenovirus, *Mycoplasma pneumoniae*, a bacterium of the genus *Salmonella*, *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Clostridium*, *Escherichia*, *Klebsiella*, *Vibrio*, *Mycobacterium*, amoeba, a malarial parasite, *Trypanosoma cruzi*, etc.

[0045] Immunogens may be obtained by isolation directly from a neoplasm, an infected cell, a specimen from an infected subject, a cell culture, or an organism culture, or may be synthesized by chemical or recombinant techniques. By way of non-limiting examples, suitable antigenic peptides, particularly for use in a hybrid antigen, for use against viruses, bacteria and the like can be designed by searching through their sequences for MHC class I restricted peptide epitopes containing HLA binding sequences such as but not limited to HLA-A2 peptide binding sequences:

Xaa(Leu/Met)XaaXaaXaa(Val/Ile/Leu/Thr)XaaXaa(Val/Leu) (SEQ ID NO:2), for example,

from viruses:

Ser Gly Pro Ser Asn Thr Pro Pro Glu Ile (SEQ ID NO:31);

Ser Gly Val Glu Asn Pro Gly Gly Tyr Cys Leu (SEQ ID NO:32);

Lys Ala Val Tyr Asn Phe Ala Thr Cys Gly (SEQ ID NO:33);

Arg Pro Gln Ala Ser Gly Val Tyr Met (SEQ ID NO:34);

Phe Gln Pro Gln Asn Gly Gln Phe Ile (SEQ ID NO:35);

Ile Glu Gly Gly Trp Thr Gly Met Ile (SEQ ID NO:36);

Thr Tyr Val Ser Val Ser Thr Ser Thr Leu (SEQ ID NO:37);

Phe Glu Ala Asn Gly Asn Leu Ile (SEQ ID NO:38);

Ile Tyr Ser Thr Val Ala Ser Ser Leu (SEQ ID NO:39);

Thr Tyr Gln Arg Thr Arg Ala Leu Val (SEQ ID NO:40);

Cys Thr Glu Leu Lys Leu Ser Asp Tyr (SEQ ID NO:41);

Ser Asp Tyr Glu Gly Arg Leu Ile (SEQ ID NO:42);

Glu Glu Gly Ala Ile Val Gly Glu Ile (SEQ ID NO:43);

Val Ser Asp Gly Gly Pro Asn Leu Tyr (SEQ ID NO:44);

Ala Ser Asn Glu Asn Met Glu Thr Met (SEQ ID NO:45);

Ala Ser Asn Glu Asn Met Asp Ala Met (SEQ ID NO:46);

Lys Leu Gly Glu Phe Tyr Asn Gln Met Met (SEQ ID NO:47);

Leu Tyr Gln Asn Val Gly Thr Tyr Val (SEQ ID NO:48);

Thr Tyr Val Ser Val Gly Thr Ser Thr Leu (SEQ ID NO:49);

Phe Glu Ser Thr Gly Asn Leu Ile (SEQ ID NO:50);

Val Tyr Gln Ile Leu Ala Ile Tyr Ala (SEQ ID NO:51);

Ile Tyr Ala Thr Val Ala Gly Ser Leu (SEQ ID NO:52);

Gly Ile Leu Gly Phe Val Phe Thr Leu (SEQ ID NO:53);

Ile Leu Gly Phe Val Phe Thr Leu Thr Val (SEQ ID NO:54);

Ile Leu Arg Gly Ser Val Ala His Lys (SEQ ID NO:55);

Glu Asp Leu Arg Val Leu Ser Phe Ile (SEQ ID NO:56);

Glu Leu Arg Ser Arg Tyr Trp Ala Ile (SEQ ID NO:57);

Ser Arg Tyr Trp Ala Ile Arg Thr Arg (SEQ ID NO:58);

Lys Thr Gly Gly Pro Ile Tyr Lys Arg (SEQ ID NO:59);

Phe Ala Pro Gly Asn Tyr Pro Ala Leu (SEQ ID NO:60);

Arg Arg Tyr Pro Asp Ala Val Tyr Leu (SEQ ID NO:61);

Asp Pro Val Ile Asp Arg Leu Tyr Leu (SEQ ID NO:62);
Ser Pro Gly Arg Ser Phe Ser Tyr Phe (SEQ ID NO:63);
Tyr Pro Ala Leu Gly Leu His Glu Phe (SEQ ID NO:64);
Thr Tyr Lys Asp Thr Val Gln Leu (SEQ ID NO:65);
Phe Tyr Asp Gly Phe Ser Lys Val Pro Leu (SEQ ID NO:66);
Phe Ile Ala Gly Asn Ser Ala Tyr Glu Tyr Val (SEQ ID NO:67);
Tyr Pro His Phe Met Pro Thr Asn Leu (SEQ ID NO:68);
Ala Pro Thr Ala Gly Ala Phe Phe Phe (SEQ ID NO:69);
Ser Thr Leu Pro Glu Thr Thr Val Val Arg Arg (SEQ ID NO:70);
Phe Leu Pro Ser Asp Phe Phe Pro Ser Val (SEQ ID NO:71);
Trp Leu Ser Leu Leu Val Pro Phe Val (SEQ ID NO:72);
Gly Leu Ser Pro Thr Val Trp Leu Ser Val (SEQ ID NO:73);
Asp Leu Met Gly Tyr Ile Pro Leu Val (SEQ ID NO:74);
Leu Met Gly Tyr Ile Pro Leu Val Gly Ala (SEQ ID NO:75);
Ala Ser Arg Cys Trp Val Ala Met (SEQ ID NO:76);
Lys Leu Val Ala Leu Gly Ile Asn Ala Val (SEQ ID NO:77);
Phe Leu Arg Gly Arg Ala Tyr Gly Leu (SEQ ID NO:78);
Arg Arg Ile Tyr Asp Leu Ile Glu Leu (SEQ ID NO:79);
Ile Val Thr Asp Phe Ser Val Ile Lys (SEQ ID NO:80);
Arg Arg Arg Trp Arg Arg Leu Thr Val (SEQ ID NO:81);
Glu Glu Asn Leu Leu Asp Phe Val Arg Phe (SEQ ID NO:82);
Cys Leu Gly Gly Leu Leu Thr Met Val (SEQ ID NO:83);
Ser Ser Ile Glu Phe Ala Arg Leu (SEQ ID NO:84);
Leu Tyr Arg Thr Phe Ala Gly Asn Pro Arg Ala (SEQ ID NO:85);
Asp Tyr Ala Thr Leu Gly Val Gly Val (SEQ ID NO:86);
Leu Leu Leu Gly Thr Leu Asn Ile Val (SEQ ID NO:87);
Leu Leu Met Gly Thr Leu Gly Ile Val (SEQ ID NO:88);
Thr Leu Gln Asp Ile Val Leu His Leu (SEQ ID NO:89);
Gly Leu His Cys Tyr Glu Gln Leu Val (SEQ ID NO:90);
Pro Leu Lys Gln His Phe Gln Ile Val (SEQ ID NO:91);
Arg Leu Val Thr Leu Lys Asp Ile Val (SEQ ID NO:92);
Arg Ala His Tyr Asn Ile Val Thr Phe (SEQ ID NO:93);

Leu Leu Phe Gly Tyr Pro Val Tyr Val (SEQ ID NO:94);

Ser Ala Ile Asn Asn Tyr Ala Gln Lys Leu (SEQ ID NO:95);

His Gln Ala Ile Ser Pro Arg Thr Leu (SEQ ID NO:96);

Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu (SEQ ID NO:97);

Cys Lys Gly Val Asn Lys Glu Tyr Leu (SEQ ID NO:98);

Gln Gly Ile Asn Asn Leu Asp Asn Leu (SEQ ID NO:99);

Asn Asn Leu Asp Asn Leu Arg Asp Tyr (SEQ ID NO:100);

Ser Glu Phe Leu Leu Glu Lys Arg Ile (SEQ ID NO:101);

Ser Tyr Ile Gly Ser Ile Asn Asn Ile (SEQ ID NO:102);

Ile Leu Gly Asn Lys Ile Val Arg Met Tyr (SEQ ID NO:103);

Arg Leu Arg Pro Gly Gly Lys Lys Lys (SEQ ID NO:104);

Glu Ile Lys Asp Thr Lys Glu Ala Leu (SEQ ID NO:105);

Gly Glu Ile Tyr Lys Arg Trp Ile Ile (SEQ ID NO:106);

Glu Ile Tyr Lys Arg Trp Ile Ile Leu (SEQ ID NO:107);

Arg Tyr Leu Lys Asp Gln Gln Leu Leu (SEQ ID NO:108);

Arg Gly Pro Gly Arg Ala Phe Val Thr Ile (SEQ ID NO:109);

Ile Val Gly Leu Asn Lys Ile Val Arg (SEQ ID NO:110);

Thr Val Tyr Tyr Gly Val Pro Val Trp Lys (SEQ ID NO:111);

Arg Leu Arg Asp Leu Leu Leu Ile Val Thr Arg (SEQ ID NO:112);

Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys (SEQ ID NO:113);

Ser Phe Asn Cys Gly Gly Glu Phe Phe (SEQ ID NO:114);

Gly Arg Ala Phe Val Thr Ile Gly Lys (SEQ ID NO:115);

Thr Pro Gly Pro Gly Val Arg Tyr Pro Leu (SEQ ID NO:116);

Gln Val Pro Leu Arg Pro Met Thr Tyr Lys (SEQ ID NO:117);

Thr Glu Met Glu Lys Glu Gly Lys Ile (SEQ ID NO:118);

Ile Leu Lys Glu Pro Val His Gly Val (SEQ ID NO:119);

Val Glu Ala Glu Ile Ala His Gln Ile (SEQ ID NO:120);

Arg Gly Tyr Val Tyr Gln Gly Leu (SEQ ID NO:121);

Tyr Ser Gly Tyr Ile Phe Arg Asp Leu (SEQ ID NO:122);

Val Gly Pro Val Phe Pro Pro Gly Met (SEQ ID NO:123);

Ile Ile Tyr Arg Phe Leu Leu Ile (SEQ ID NO:124);

from bacteria:

Lys Tyr Gly Val Ser Val Gln Asp Ile (SEQ ID NO:125);
Ile Gln Val Gly Asn Thr Arg Thr Ile (SEQ ID NO:126);
Thr Pro His Pro Ala Arg Ile Gly Leu (SEQ ID NO:127);
from parasites:
Ser Tyr Ile Pro Ser Ala Glu Lys Ile (SEQ ID NO:128);
Lys Pro Lys Asp Glu Leu Asp Tyr (SEQ ID NO:129);
Lys Ser Lys Asp Glu Leu Asp Tyr (SEQ ID NO:130);
Lys Pro Asn Asp Lys Ser Leu Tyr (SEQ ID NO:131);
Lys Tyr Leu Lys Lys Ile Lys Asn Ser Leu (SEQ ID NO:132);
Tyr Glu Asn Asp Ile Glu Lys Lys Ile (SEQ ID NO:133);
Asn Tyr Asp Asn Ala Gly Thr Asn Leu (SEQ ID NO:134);
Asp Glu Leu Asp Tyr Glu Asn Asp Ile (SEQ ID NO:135);
Ser Tyr Val Pro Ser Ala Glu Gln Ile (SEQ ID NO:136);
from cancers:
Phe Glu Gln Asn Thr Ala Gln Pro(SEQ ID NO:137);
Phe Glu Gln Asn Thr Ala Gln Ala (SEQ ID NO:138);
Glu Ala Asp Pro Thr Gly His Ser Tyr (SEQ ID NO:139);
Glu Val Asp Pro Ile Gly His Leu Tyr (SEQ ID NO:140);
Ala Ala Gly Ile Gly Ile Leu Thr Val (SEQ ID NO:141);
Tyr Leu Glu Pro Gly Pro Val Thr Ala (SEQ ID NO:142);
Ile Leu Asp Gly Thr Ala Thr Leu Arg Leu (SEQ ID NO:143);
Met Leu Leu Ala Leu Leu Tyr Cys Leu (SEQ ID NO:144);
Tyr Met Asn Gly Thr Met Ser Gln Val (SEQ ID NO:145);
Leu Pro Tyr Leu Gly Trp Leu Val Phe (SEQ ID NO:146);
Phe Gly Pro Tyr Lys Leu Asn Arg Leu (SEQ ID NO:147);
Lys Ser Pro Trp Phe Thr Thr Leu (SEQ ID NO:148);
Gly Pro Pro His Ser Asn Asn Phe Gly Tyr (SEQ ID NO:149); and
Ile Ser Thr Gln Asn His Arg Ala Leu (SEQ ID NO:150)
(Rammensee et al., *Immunogenetics* 41:178-223 (1995)),
Xaa(Leu/Met)XaaXaaXaaXaaXaaXaaVal (SEQ ID NO:3)
(Tarpey et al., *Immunology* 81:222-227 (1994)),
Xaa(Val/Gln)XaaXaaXaaXaaXaaXaaLeu (SEQ ID NO:28),

for example, from virus:
Tyr Gly Ile Leu Gly Lys Val Phe Thr Leu (SEQ ID NO:151);
Ser Leu Tyr Asn Thr Val Ala Thr Leu (SEQ ID NO:152);
(Barouch et al., *J. Exp. Med.* 182:1847-1856 (1995)).

[0046] The foregoing epitopes are merely exemplary of selections available associated with various infectious diseases and cancer, and are provided without any intention whatsoever to be limiting.

[0047] It may also be desirable to consider the type of immune response which is desired. For example, under certain circumstances, a humoral immune response may be appropriate. In other cases, and indeed where an immune response directed toward neoplastic cells or infected cells is sought to be elicited, a cellular immune response is particularly desirable. Accordingly, particular epitopes associated with the activation of B cells, T helper cells, or cytotoxic T cells may be identified and selected for incorporation into the hybrid antigen.

[0048] It may also be desirable to utilize hybrid antigen associated with an autoimmune disease or allergy. Such a hybrid antigen may be administered, together with one or more heat shock proteins, in an amount sufficient to be tolerogenic or to inhibit a pre-existing immune response to the hybrid antigen in a subject. The amount of heat shock protein required to inhibit the immune response is expected to be substantially greater than the amount required for stimulation.

[0049] Although the size of hybrid antigen may vary depending upon the heat shock protein used, in non-limiting embodiments of the invention, the hybrid antigen may be the size of a peptide having between 10 and 500 amino acid residues, and preferably be the size of a peptide having between 14 and 100, most preferably 18 and 50 amino acid residues. As such, it may be desirable to produce a fragment of an immunogen to serve as the antigenic domain of a hybrid antigen, or, alternatively, to synthesize a hybrid antigen by chemical or recombinant DNA methods.

[0050] Based on the foregoing considerations, a hybrid antigen may be prepared, and then tested for its ability to bind to heat shock protein. In some instances, binding of hybrid

antigen to a particular heat shock protein may be facilitated by the presence of at least one other protein, which may be a heat shock protein.

[0051] For example, binding of hybrid antigen to a heat shock protein may be evaluated by labeling the hybrid antigen with a detectable label, such as a radioactive, fluorescent, enzymatic or pigmented label, combining the hybrid antigen with heat shock protein under conditions which would be expected to permit binding to occur, and then isolating the heat shock protein while removing any unbound hybrid antigen, and determining whether any labeled hybrid antigen had adhered to the heat shock protein. As a specific example, and not by way of limitation, the ability of a hybrid antigen to bind to the heat shock protein BiP may be evaluated by combining 2 μg BiP with up to about 1150 pmole of radioactively labeled hybrid antigen in buffer containing 50 mM Tris HCl (pH 7.5), 200 mM NaCl, and 1 mM Na_2EDTA, in a final volume of 50 μl, for 30 minutes at 37 degrees Centigrade. Unbound hybrid antigen may then be removed from bound BiP-hybrid antigen by centrifugation at 100g by desalting through a 1 ml Sephadex-G column for 2 minutes. Penefsky, *J. Biol. Chem.* 252:2891 (1977). To prevent binding to the resin, columns may first be treated with 100 μl of bovine serum albumin in the same buffer and centrifuged as above. Bound hybrid antigen may then be quantitated by liquid scintillation counting. See Flynn et al., *Science* 245:385-390 (1989).

[0052] Because ATP hydrolysis drives the release of peptides from many known heat shock proteins, the amount of ATPase activity may often be used to quantitate the amount of hybrid antigen binding to heat shock protein. An example of how such an assay may be performed is set forth in Flynn et al., *Science* 245:385-390 (1989).

[0053] The heat shock protein-binding domain is selected so that the hybrid antigen will bind *in vitro* or *in vivo* to a heat shock protein such as BiP, hsp70, gp96, or hsp90, or a member of the foregoing heat shock protein families, alone or in combination with accessory heat shock proteins such as hsp40, or hsp60.

[0054] Non-limiting examples of peptides which fulfill this criterion may be identified by panning libraries of antigens known to bind well to one or more heat shock proteins as described in Blond-Elguindi et al., *Cell* 75:717-728 (1993):

Leu Phe Trp Pro Phe Glu Trp Ile (SEQ ID NO:153);

Asp Gly Val Gly Ser Phe Ile Gly (SEQ ID NO:154);
Glu Ser Leu Trp Asn Pro Gln Cys (SEQ ID NO:155);
Leu His Phe Asp Val Leu Trp Arg (SEQ ID NO:156);
Cys His Leu Lys Met Val Pro Trp (SEQ ID NO:157);
Asn Ser Val Leu Val Cys Glu Leu (SEQ ID NO:158);
Asp Arg Gly His Ser Thr Tyr Ser (SEQ ID NO:159);
Asp Val Trp Gly Trp Val Thr Trp (SEQ ID NO:160);
Ile Gln Phe Arg Val Glu Leu Phe (SEQ ID NO:161);
Leu Trp Leu Glu Leu Ser Leu Ser (SEQ ID NO:162);
Val Gly Ile Cys Ala Leu Phe Gly (SEQ ID NO:163);
Pro Tyr Pro Ser Gly Leu Asp Ser (SEQ ID NO:164);
Phe Trp Gly Val Leu Pro Tyr Pro (SEQ ID NO:165);
Phe Thr His Gly Ile Ser Leu Tyr (SEQ ID NO:166);
Asn His Ser Phe Gly Gly Ser Thr (SEQ ID NO:167);
Val Asp Tyr Val Tyr Phe His His (SEQ ID NO:168);
Phe Leu Asp Ile Ile Gly Tyr Gly (SEQ ID NO:169);
Trp Asp Asp Leu Leu His Gly Arg (SEQ ID NO:170);
Leu Arg Leu Leu Gly Thr Leu Asn (SEQ ID NO:171);
Phe Glu Gln His Asn Gln Glu Pro (SEQ ID NO:172);
Phe Val Gly Thr Val Thr Trp Ser (SEQ ID NO:173);
Leu Trp Ala Leu Thr Tyr Arg Gly (SEQ ID NO:174);
Ser Trp Gly Ser Asn Gly Gly Phe (SEQ ID NO:175);
Asp Met Trp Arg Arg Ala Val Gln (SEQ ID NO:176);
Cys Arg Val Ile Tyr His Ala Thr (SEQ ID NO:177);
Met Val Val Ala Arg Cys Gly His (SEQ ID NO:178);
His Met Trp Ile Asn Trp Val Gln (SEQ ID NO:179);
Cys Ala Gly Arg Cys Phe Gly Tyr (SEQ ID NO:180);
Cys Thr His Val Leu Ala Tyr Ser (SEQ ID NO:181);
Ser Trp Met Pro Trp Leu Thr Met (SEQ ID NO:182);
Leu Glu Trp Cys Ile Trp Arg Tyr (SEQ ID NO:183);
Cys Leu Ala Cys Ile Ile His Ser (SEQ ID NO:184);
Phe Trp Phe Pro Trp Asp Arg Ser (SEQ ID NO:185);

Trp Arg Thr Gly Val Phe His Gly (SEQ ID NO:186);
Met His Leu Arg Val Ala Asp Arg (SEQ ID NO:187);
Ala Leu Asp Leu Tyr Leu Tyr Val (SEQ ID NO:188);
Phe Phe Trp Phe Thr Leu Lys Glu (SEQ ID NO:189);
Leu Ser Phe Ala Gly Trp Gly Val (SEQ ID NO:190);
Met Met Met Leu Gly Arg Ala Pro (SEQ ID NO:191);
Trp Ser Phe Tyr Thr Trp Leu Asn (SEQ ID NO:192);
Phe Val Trp Met Arg Trp Ile Asp (SEQ ID NO:193);
Met Gln Val Asn Thr Pro Asp Asn (SEQ ID NO:194);
Phe Trp Gly Trp Leu Ile Pro Trp (SEQ ID NO:195);
Trp Gly Trp Val Trp Trp Asp (SEQ ID NO:196);
Trp Ile Phe Pro Trp Ile Gln Leu (SEQ ID NO:197);
Trp Met Phe Asn Trp Pro Trp Tyr (SEQ ID NO:198);
Met Asn Met Ile Val Leu Asp Lys (SEQ ID NO:199);
Phe Trp Gly Trp Pro Gly Trp Ser (SEQ ID NO:200);
Trp Leu Ile Arg Val Gly Thr Ala (SEQ ID NO:201);
Gly Leu Leu Thr His Leu Ile Trp (SEQ ID NO:202);
Leu Trp Trp Leu Asn Val His Gly (SEQ ID NO:203);
Trp Trp Trp Ile Asn Asp Glu Ser (SEQ ID NO:204);
Ala Asn Pro Ser Leu Ala Thr Tyr (SEQ ID NO:205);
Trp Leu Gln Gly Trp Trp Gly Trp (SEQ ID NO:206);
Met Met Pro Val Thr Ser Phe Arg (SEQ ID NO:207);
Gly Trp Met Asp Trp Trp Tyr Tyr (SEQ ID NO:208);
Leu Ala Ser Met Arg Asn Ser Met (SEQ ID NO:209);
Asp Leu Met Arg Trp Leu Gly Leu (SEQ ID NO:210);
Tyr Phe Tyr Ala Trp Trp Leu Asp (SEQ ID NO:211);
Leu Gly His Leu Trp Thr Gln Val (SEQ ID NO:212);
Leu Trp Trp Arg Asp Val Met Ala (SEQ ID NO:213);
Phe Ile Trp Trp Ala Pro Leu Ala (SEQ ID NO:214);
Gly Ser Val Gly Gly Gly Val Val (SEQ ID NO:215);
Asp Ser His Asp Asp Trp Arg Met (SEQ ID NO:216);
Phe Trp Arg Phe Asp Tyr Tyr Phe (SEQ ID NO:217);

Trp Thr Trp Trp Glu Trp Leu Ala (SEQ ID NO:218);
Trp Leu Trp Asp Trp Ile Val Val (SEQ ID NO:219);
Gly Trp Thr Trp Phe Phe Asp Met (SEQ ID NO:220);
Ala Trp Trp Gln His Phe Ile Val (SEQ ID NO:221);
Leu Trp Trp Asp Ile Ile Thr Gly (SEQ ID NO:222);
Phe Thr Tyr Gly Ser Arg Trp Leu (SEQ ID NO:223);
Phe Ser Leu Trp Pro Leu Ala Trp (SEQ ID NO:224);
Gly Ile Ile Leu Gly Tyr Asn Val (SEQ ID NO:225);
Ser Trp Met Thr Trp Ile Glu His (SEQ ID NO:226);
Gly Trp Trp Val Thr Trp Pro Trp (SEQ ID NO:227);
Val Val Ser Pro Trp Trp Leu Gly (SEQ ID NO:228);
Asn Val Leu Ser Arg Gly Phe Ser (SEQ ID NO:229);
Ser Phe Glu Ser Leu Gly Gly Leu (SEQ ID NO:230);
Ile Thr Lys Gly Ser Ser Phe Pro (SEQ ID NO:231);
Leu Asp Trp Ala Arg Lys Leu Arg (SEQ ID NO:232);
Thr Ala Trp Asn Leu Leu Gly Tyr (SEQ ID NO:233);
Phe Gly Gln Gly Ile Lys His Val (SEQ ID NO:234);
Asp Val Val Trp Gln Arg Leu Leu (SEQ ID NO:235);
Tyr Val Asp Arg Phe Ile Gly Trp (SEQ ID NO:236);
Lys Met Ala Arg Pro Glu Gly Asn (SEQ ID NO:237);
Leu Gly Arg Trp Gly His Glu Ser (SEQ ID NO:238);
Ser Ile Trp Ser Leu Leu Val Leu (SEQ ID NO:239);
Val Trp Leu Asp Leu Leu Leu Ser (SEQ ID NO:240);
Tyr Leu Thr Asp Ser Leu Phe Gly (SEQ ID NO:241);
Thr Trp Trp Pro Ser Ile Thr Trp (SEQ ID NO:242);
Tyr Gly Leu Trp Trp Phe Pro Trp (SEQ ID NO:243);
Phe Ser Pro Ala Asp Thr Arg Tyr (SEQ ID NO:244);
Cys Asn Arg Leu Gln Ile Asp Cys (SEQ ID NO:245);
Ser Leu Val Ala Ala Arg Asn Leu (SEQ ID NO:246);
Phe Thr Ile His Asn Val Ala Val (SEQ ID NO:247);
Met Gly Pro Leu Gly Pro Leu Leu (SEQ ID NO:248);
Arg Gln Leu Ser Glu Leu Phe Val (SEQ ID NO:249);

Arg Val Val Cys Gln Ala Leu Leu (SEQ ID NO:250);
Trp Pro His Leu Trp Trp Leu Asp (SEQ ID NO:251);
Trp Met Asp Trp Val Trp His Thr (SEQ ID NO:252);
Trp Trp Gly Tyr Leu Ile Cys Gln (SEQ ID NO:253);
Phe Arg Gly Leu Ser Glu Gly Pro (SEQ ID NO:254);
Ser Trp Phe Asp Trp Leu Val Ala (SEQ ID NO:255);
Val Val Met Trp Tyr Ser Val Asp (SEQ ID NO:256);
Trp Gly Trp Ser Leu Ala Thr (SEQ ID NO:257);
Leu Gly Trp Phe Asp Arg Phe Phe (SEQ ID NO:258);
Ala Trp Trp Trp Pro Thr Tyr Val (SEQ ID NO:259);
Gly Phe Leu Ser Ser Trp Phe Leu (SEQ ID NO:260);
Gly Val Ile Asn Cys Ala Gly Thr (SEQ ID NO:261);
Val Cys Ala Arg Ala Ala His Leu (SEQ ID NO:262);
Gly Asn Ser Tyr Gly Asp Gly Gly (SEQ ID NO:263);
Gly Phe Leu Ser Ser Trp Phe Leu (SEQ ID NO:264);
Phe Asp Gln Pro Gly Arg Phe Leu (SEQ ID NO:265);
Arg Ser His Ala Thr Gly Val Val (SEQ ID NO:266);
Gly Tyr Trp Ala Met Met Ser Trp (SEQ ID NO:267);
Cys His Ser Met Trp Asp Gly Leu (SEQ ID NO:268);
Phe Ile Trp Arg Gly Trp Pro His (SEQ ID NO:269);
Leu Ser Phe Leu Gly Gly Arg Leu (SEQ ID NO:270);
Phe Ser Gly Val Arg Gln Pro Asn (SEQ ID NO:271);
Trp Gly Trp Met Pro Phe Tyr Tyr (SEQ ID NO:272);
Phe Thr Arg Pro Ala Val Val Asp (SEQ ID NO:273);
Asp Leu Trp Thr Trp Leu Gly Leu (SEQ ID NO:274);
Cys Asp Thr Ala Ala Val Ala Asp (SEQ ID NO:275);
Trp Trp Val Lys His His Met Leu (SEQ ID NO:276);
Ile Ala Phe Leu Arg Asp Asn Arg (SEQ ID NO:277);
Leu Ala Arg Pro Asp His Tyr Ser (SEQ ID NO:278);
Met Glu Ser Lys Arg Trp Thr Val (SEQ ID NO:279);
Met Ile Leu Lys Gly Tyr Ser Arg (SEQ ID NO:280);
Ala Pro Ser Asp Tyr Asp Glu Ser (SEQ ID NO:281);

His Trp Leu Arg Ser Lys Arg Thr (SEQ ID NO:282);
Gly Ala Arg Val Trp Asn Tyr Gln (SEQ ID NO:283);
Leu Ser Asn Trp Asn Met Arg Leu (SEQ ID NO:284);
Cys Gly Ala Ala Gln Gln Gly Met (SEQ ID NO:285); and
Gly Ser Ser Met Val Val Gln Arg (SEQ ID NO:286). Using this technique, Blond-Elguindi have concluded that the heat shock protein BiP recognizes polypeptides that contain a heptameric region having the sequence

Hy(Trp/X)HyXHyXHy (SEQ ID NO: 29)

where Hy represents a hydrophobic amino acid residue, particularly tryptophan, leucine or phenylalanine (SEQ ID NO:30), and X is any amino acid. High affinity heat-shock protein-binding sequences incorporating this motif include:
His Trp Asp Phe Ala Trp Pro Trp (SEQ ID NO:1); and
Phe Trp Gly Leu Trp Pro Trp Glu (SEQ ID NO:4).

[0055] Other heat shock protein binding motifs have also been identified. For example, Auger et al., *Nature Medicine* 2:306-310 (1996) have identified two pentapeptide binding motifs

Gln Lys Arg Ala Ala (SEQ ID NO:5) and
Arg Arg Arg Ala Ala (SEQ ID NO:6)

in HLA-DR types associated with rheumatoid arthritis which bind to heat shock proteins. Heat shock binding motifs have also been identified as consisting of seven to fifteen residue long peptides which are enriched in hydrophobic amino acids.
Lys Arg Gln Ile Tyr Asp Leu Glu Met Asn Arg Leu Gly Lys (SEQ ID NO:287);
Leu Ser Ser Leu Phe Arg Pro Lys Arg Arg Pro Ile Tyr Lys Ser (SEQ ID NO:288);
Lys Leu Ile Gly Val Leu Ser Ser Leu Phe Arg Pro Lys (SEQ ID NO:289);
Arg Arg Pro Ile Tyr Lys Ser Asp Val Gly Met Ala His Phe Arg (SEQ ID NO:290);
Cys Lys Ile Gln Ser Thr Pro Val Lys Gln Ser (SEQ ID NO:291);
Tyr His Cys Asp Gly Phe Gln Asn Glu (SEQ ID NO:292);
Val Gly Ile Asp Leu Gly Thr Thr Tyr Ser Cys (SEQ ID NO:293);
Ser Asn Gly Ser Leu Gln Cys Arg Ile Cys (SEQ ID NO:294)
(Flynn et al., *Science* 245: 385-390 (1989)),

[0056] Moreover, other heat shock protein binding peptides include:

Gly Lys Trp Val Tyr Ile (SEQ ID NO:295);

Ala Lys Arg Glu Thr Lys (SEQ ID NO:296);

Lys Trp Val His Leu Phe (SEQ ID NO:297);

Arg Leu Val Leu Val Leu (SEQ ID NO:298);

Trp Lys Trp Gly Ile Tyr (SEQ ID NO:299);

Ser Ser His Ala Ser Ala (SEQ ID NO:300);

Trp Gly Pro Trp Ser Phe (SEQ ID NO:301);

Ala Ile Pro Gly Lys Val (SEQ ID NO:302);

Arg Val His Asp Pro Ala (SEQ ID NO:303);

Arg Ser Val Ser Ser Phe (SEQ ID NO:304);

Leu Gly Thr Arg Lys Gly (SEQ ID NO:305);

Lys Asp Pro Leu Phe Asn (SEQ ID NO:306);

Leu Ser Gln His Thr Asn (SEQ ID NO:307);

Asn Arg Leu Leu Leu Thr (SEQ ID NO:308);

Tyr Pro Leu Trp Val Ile (SEQ ID NO:309);

Leu Leu Ile Ile Asp Arg (SEQ ID NO:310);

Arg Val Ile Ser Leu Gln (SEQ ID NO:311);

Glu Val Ser Arg Glu Asp (SEQ ID NO:312);

Ser Ile Leu Arg Ser Thr (SEQ ID NO:313);

Pro Gly Leu Val Trp Leu (SEQ ID NO:314);

Val Lys Lys Leu Tyr Ile (SEQ ID NO:315);

Asn Asn Arg Leu Leu Asp (SEQ ID NO:316);

Ser Lys Gly Arg Trp Gly (SEQ ID NO:317);

Ile Arg Pro Ser Gly Ile (SEQ ID NO:318);

Ala Ser Leu Cys Pro Thr (SEQ ID NO:319);

Asp Val Pro Gly Leu Arg (SEQ ID NO:320);

Arg His Arg Glu Val Gln (SEQ ID NO:321);

Leu Ala Arg Lys Arg Ser (SEQ ID NO:322);

Ser Val Leu Asp His Val (SEQ ID NO:323);

Asn Leu Leu Arg Arg Ala (SEQ ID NO:324);

Ser Gly Ile Ser Ala Trp (SEQ ID NO:325);

Phe Tyr Phe Trp Val Arg (SEQ ID NO:326);

Lys Leu Phe Leu Pro Leu (SEQ ID NO:327);

Thr Pro Thr Leu Ser Asp (SEQ ID NO:328);

Thr His Ser Leu Ile Leu (SEQ ID NO:329);

Leu Leu Leu Leu Ser Arg (SEQ ID NO:330);

Leu Leu Arg Val Arg Ser (SEQ ID NO:331);

Glu Arg Arg ser Arg Gly (SEQ ID NO:332);

Arg Met Leu Gln Leu Ala (SEQ ID NO:333);

Age Gly Trp Ala Asn Ser (SEQ ID NO:334);

Arg Pro Phe Tyr Ser Tyr (SEQ ID NO:335);

Ser Ser Ser Trp Asn Ala (SEQ ID NO:336);

Leu Gly His Leu Glu Glu (SEQ ID NO:337);

Ser Ala Val Thr Asn Thr (SEQ ID NO:338);

Leu Arg Arg Ala Ser Leu (SEQ ID NO:339);

Leu Arg Arg Trp Ser Leu (SEQ ID NO:340);

Lys Trp Val His Leu Phe (SEQ ID NO:341);

Asn Arg Leu Leu Leu Thr (SEQ ID NO:342);

Ala Arg Leu Leu Leu Thr (SEQ ID NO:343);

Asn Ala Leu Leu Leu Thr (SEQ ID NO:344);

Asn Arg Leu Ala Leu Thr (SEQ ID NO:345);

Asn Leu Leu Arg Leu Thr (SEQ ID NO:346);

Asn Arg Leu Trp Leu Thr (SEQ ID NO:347);

Asn Arg Leu Leu Leu Ala (SEQ ID NO:348);

Met Gln Glu Arg Ile Thr Leu Lys Asp Tyr Ala Met (SEQ ID NO:349);

Leu Arg Arg Trp Ser Leu Gly (SEQ ID NO:353);

Lys Trp Val His Leu Phe Gly (SEQ ID NO:354);

Asn Arg Leu Leu Leu Thr Gly (SEQ ID NO:355);

Ala Arg Leu Leu Leu Thr Gly (SEQ ID NO:356);

Asn Ala Leu Leu Leu Thr Gly (SEQ ID NO:357);

Asn Arg Leu Ala Leu Thr Gly (SEQ ID NO:358);

Asn Leu Leu Arg Leu Thr Gly (SEQ ID NO:359);

Asn Arg Leu Trp Leu Thr Gly (SEQ ID NO:360);

Asn Arg Leu Leu Leu Ala Gly (SEQ ID NO:361);
Gly Lys Trp Val Tyr Ile Gly (SEQ ID NO:295);
Ala Lys Arg Glu Thr Lys Gly (SEQ ID NO:296);
Lys Trp Val His Leu Phe Gly (SEQ ID NO:297);
Arg Leu Val Leu Val Leu Gly (SEQ ID NO:298);
Trp Lys Trp Gly Ile Tyr (SEQ ID NO:299);
Ser Ser His Ala Ser Ala (SEQ ID NO:300);
Trp Gly Pro Trp Ser Phe (SEQ ID NO:301);
Ala Ile Pro Gly Lys Val (SEQ ID NO:302);
Arg Val His Asp Pro Ala Gly (SEQ ID NO:303);
Arg Ser Val Ser Ser Phe Gly (SEQ ID NO:304);
Leu Gly Thr Arg Lys Gly Gly (SEQ ID NO:305);
Lys Asp Pro Leu Phe Asn Gly (SEQ ID NO:306);
Leu Ser Gln His Thr Asn Gly (SEQ ID NO:307);
Asn Arg Leu Leu Leu Thr Gly (SEQ ID NO:308);
Tyr Pro Leu Trp Val Ile Gly (SEQ ID NO:309);
Leu Leu Ile Ile Asp Arg Gly (SEQ ID NO:310);
Arg Val Ile Ser Leu Gln Gly (SEQ ID NO:311);
Glu Val Ser Arg Glu Asp Gly (SEQ ID NO:312);
Ser Ile Leu Arg Ser Thr Gly (SEQ ID NO:313);
Pro Gly Leu Val Trp Leu Gly (SEQ ID NO:314);
Val Lys Lys Leu Tyr Ile Gly (SEQ ID NO:315);
Asn Asn Arg Leu Leu Asp Gly (SEQ ID NO:316);
Ser Lys Gly Arg Trp Gly Gly (SEQ ID NO:317);
Ile Arg Pro Ser Gly Ile Gly (SEQ ID NO:318);
Ala Ser Leu Cys Pro Thr Gly (SEQ ID NO:319);
Asp Val Pro Gly Leu Arg Gly (SEQ ID NO:320);
Arg His Arg Glu Val Gln Gly (SEQ ID NO:321);
Leu Ala Arg Lys Arg Ser Gly (SEQ ID NO:322);
Ser Val Leu Asp His Val Gly (SEQ ID NO:323);
Asn Leu Leu Arg Arg Ala Gly (SEQ ID NO:324);
Ser Gly Ile Ser Ala Trp Gly (SEQ ID NO:325);

Phe Tyr Phe Trp Val Arg Gly (SEQ ID NO:326);
Lys Leu Phe Leu Pro Leu Gly (SEQ ID NO:327);
Thr Pro Thr Leu Ser Asp Gly (SEQ ID NO:328);
Thr His Ser Leu Ile Leu Gly (SEQ ID NO:329);
Leu Leu Leu Leu Ser Arg Gly (SEQ ID NO:330);
Leu Leu Arg Val Arg Ser Gly (SEQ ID NO:331);
Glu Arg Arg ser Arg Gly Gly (SEQ ID NO:332);
Arg Met Leu Gln Leu Ala Gly (SEQ ID NO:333);
Age Gly Trp Ala Asn Ser Gly (SEQ ID NO:334);
Arg Pro Phe Tyr Ser Tyr Gly (SEQ ID NO:335);
Ser Ser Ser Trp Asn Ala Gly (SEQ ID NO:336);
Leu Gly His Leu Glu Glu Gly (SEQ ID NO:337); and
Ser Ala Val Thr Asn Thr Gly (SEQ ID NO:338); as described by Gragerov et al., J. Molec. Biol. 235:848-854 (1994).

[0057] Other heat shock protein binding domains include Phe Tyr Gln Leu Ala Leu Thr(SEQ ID NO:), Phe Tyr Gln Leu Ala Leu Thr Trp (SEQ ID NO:), Arg Lys Leu Phe Phe Asn Leu Arg (SEQ ID NO:), Arg Lys Leu Phe Phe Asn Leu Arg Trp (SEQ ID NO:), Lys Phe Glu Arg Gln (SEQ ID NO:), Asn Ile Val Arg Lys Lys Lys (SEQ ID NO:), and Arg Gly Tyr Val Tyr Gln Gly Leu (SEQ ID NO:).

[0058] Moreover, other heat shock protein binding domains include those described in WO9922761. Xaa represents any amino acid.

HTTVYGAG (SEQ ID NO: 82);
TETPYPTG (SEQ ID NO: 83);
LTTPFSSG (SEQ ID NO: 84);
GVPLTMDG (SEQ ID NO: 85);
KLPTVLRG (SEQ ID NO: 86);
CRFHGNRG (SEQ ID NO: 87);
YTRDFEAG (SEQ ID NO: 88);
SSAAGPRG (SEQ ID NO: 89);
SLIQYSRG (SEQ ID NO: 90);

DALMWP Xaa G (SEQ ID NO: 91);
SS Xaa SLYIG (SEQ ID NO: 92);
FNTSTRTG (SEQ ID NO: 93);
TVQHVAFG (SEQ ID NO: 94);
DYSFPPLG (SEQ ID NO: 95);
VGSMESLG (SEQ ID NO: 96);
F Xaa PMI Xaa SG (SEQ ID NO: 97);
APPRVTMG (SEQ ID NO: 98);
IATKTPKG (SEQ ID NO: 99);
KPPLFQIG (SEQ ID NO: 100);
YHTAHNMG (SEQ ID NO: 101);
SYIQATHG (SEQ ID NO: 102);
SSFATFLG (SEQ ID NO: 103);
TTPPNFAG (SEQ ID NO: 104);
ISLDPRMG (SEQ ID NO: 105);
SLPLFGAG (SEQ ID NO: 106);
NLLKTTLG (SEQ ID NO: 107);
DQNLPRRG (SEQ ID NO: 108);
SHFEQLLG (SEQ ID NO: 109);
TPQLHHGG (SEQ ID NO: 110);
APLDRITG (SEQ ID NO: 111);
FAPLIAHG (SEQ ID NO: 112);
SWIQTFMG (SEQ ID NO: 113);
NTWPHMYG (SEQ ID NO: 114);
EPLPTTLG (SEQ ID NO: 115);
HGPHLFNG (SEQ ID NO: 116);
YLNSTLAG (SEQ ID NO: 117);
HLHSPSGG (SEQ ID NO: 118);
TLPHRLNG (SEQ ID NO: 119);
SSPREVHG (SEQ ID NO: 120);
NQVDTARG (SEQ ID NO: 121);
YPTPLLTG (SEQ ID NO: 122);

HPAAFPWG (SEQ ID NO: 123);
LLPHSSAG (SEQ ID NO: 124);
LETYTASG (SEQ ID NO: 125);
KYVPLPPG (SEQ ID NO: 126);
APLALHAG (SEQ ID NO: 127);
YESLLTKG (SEQ ID NO: 128);
SHAASGTG (SEQ ID NO: 129);
GLATVKSG (SEQ ID NO: 130);
GATSFGLG (SEQ ID NO: 131);
KPPGPVSG (SEQ ID NO: 132);
TLYVSGNG (SEQ ID NO: 133);
HAPFKSQG (SEQ ID NO: 134);
VAFTRLPG (SEQ ID NO: 135);
LPTRTPAG (SEQ ID NO: 136);
ASFDLLIG (SEQ ID NO: 137);
RMNTEPPG (SEQ ID NO: 138);
KMTPLTTG (SEQ ID NO: 139);
ANATPLLG (SEQ ID NO: 140);
TIWPPPVG (SEQ ID NO: 141);
QTKVMTTG (SEQ ID NO: 142);
NHAVFASG (SEQ ID NO: 143);
LHAA Xaa TSG (SEQ ID NO: 144);
TWQPYFHG (SEQ ID NO: 145);
APLALHAG (SEQ ID NO: 146);
TAHDLTVG (SEQ ID NO: 147);
NMTNMLTG (SEQ ID NO: 148);
GSGLSQDG (SEQ ID NO: 149);
TPIKTIYG (SEQ ID NO: 150);
SHLYRSSG (SEQ ID NO: 151);
YTLVQPL (SEQ ID NO: 152);
TPDITPK (SEQ ID NO: 153);
TYPDLRY (SEQ ID NO: 154);

DRTHATS (SEQ ID NO: 155);
MSTTFYS (SEQ ID NO: 156);
YQHAVQT (SEQ ID NO: 157);
FPFSAST (SEQ ID NO: 158);
SSFPPLD (SEQ ID NO: 159);
MAPSPPH (SEQ ID NO: 160);
SSFPDLL (SEQ ID NO: 161);
HSYNRLP (SEQ ID NO: 162);
HLTHSQR (SEQ ID NO: 163);
QAAQSRS (SEQ ID NO: 164);
FATHHIG (SEQ ID NO: 165);
SMPEPLI (SEQ ID NO: 166);
IPRYHLI (SEQ ID NO: 167);
SAPHMTS (SEQ ID NO: 168);
KAPVWAS (SEQ ID NO: 169);
LPHWLLI (SEQ ID NO: 170);
ASAGYQI (SEQ ID NO: 171);
VTPKTGS (SEQ ID NO: 172);
EHPMPVL (SEQ ID NO: 173);
VSSFVTS (SEQ ID NO: 174);
STHFTWP (SEQ ID NO: 175);
GQWWSPD (SEQ ID NO: 176);
GPPHQDS (SEQ ID NO: 177);
NTLPSTI (SEQ ID NO: 178);
HQPSRWV (SEQ ID NO: 179);
YGNPLQP (SEQ ID NO: 180);
FHWWWQP (SEQ ID NO: 181);
ITLKYPL (SEQ ID NO: 182);
FHWPWLF (SEQ ID NO: 183);
TAQDSTG (SEQ ID NO: 184);
FHWWWQP (SEQ ID NO: 185);
FHWWDWW (SEQ ID NO: 186);

EPFFRMQ (SEQ ID NO: 187);
TWWLNYR (SEQ ID NO: 188);
FHWWWQP (SEQ ID NO: 189);
QPSHLRW (SEQ ID NO: 190);
SPASPVY (SEQ ID NO: 191);
FHWWWQP (SEQ ID NO: 192);
HPSNQAS (SEQ ID NO: 193);
NSAPRPV (SEQ ID NO: 194);
QLWSIYP (SEQ ID NO: 195);
SWPFFDL (SEQ ID NO: 196);
DTTLPLH (SEQ ID NO: 197);
WHWQMLW (SEQ ID NO: 198);
DSFRTPV (SEQ ID NO: 199);
TSPLSLL (SEQ ID NO: 200);
AYNYVSD (SEQ ID NO: 201);
RPLHDPM (SEQ ID NO: 202);
WPSTTLF (SEQ ID NO: 203);
ATLEPVR (SEQ ID NO: 204);
SMTVLRP (SEQ ID NO: 205);
QIGAPSW (SEQ ID NO: 206);
APDLYVP (SEQ ID NO: 207);
RMPPLLP (SEQ ID NO: 208);
AKATPEH (SEQ ID NO: 209);
TPPLRIN (SEQ ID NO: 210);
LPIHAPH (SEQ ID NO: 211);
DLNAYTH (SEQ ID NO: 212);
VTLPNFH (SEQ ID NO: 213);
NSRLPTL (SEQ ID NO: 214);
YPHPSRS (SEQ ID NO: 215);
GTAHFMY (SEQ ID NO: 216);
YSLLPTR (SEQ ID NO: 217);
LPRRTLL (SEQ ID NO: 218);

TSTLLWK (SEQ ID NO: 219);
TSDMKPH (SEQ ID NO: 220);
TSSYLAL (SEQ ID NO: 221);
NLYGPHD (SEQ ID NO: 222);
LETYTAS (SEQ ID NO: 223);
AYKSLTQ (SEQ ID NO: 224);
STSVYSS (SEQ ID NO: 225);
EGPLRSP (SEQ ID NO: 226);
TTYHALG (SEQ ID NO: 227);
VSIGHPS (SEQ ID NO: 228);
THSHRPS (SEQ ID NO: 229);
ITNPLTT (SEQ ID NO: 230);
SIQAHHS (SEQ ID NO:231);
LNWPRVL (SEQ ID NO:232);
YYYAPPP (SEQ ID NO:233);
SLWTRLP (SEQ ID NO:234);
NVYHSSL (SEQ ID NO:235);
NSPHPPT (SEQ ID NO:236);
VPAKPRH (SEQ ID NO:237);
HNLHPNR (SEQ ID NO:238);
YTTHRWL (SEQ ID NO:239);
AVTAAIV (SEQ ID NO:240);
TLMHDRV (SEQ ID NO:241);
TPLKVPY (SEQ ID NO:242);
FTNQQYH (SEQ ID NO:243);
SHVPSMA (SEQ ID NO:244);
HTTVYGA (SEQ ID NO:245);
TETPYPT (SEQ ID NO:246);
LTTPFSS (SEQ ID NO:247);
GVPLTMD (SEQ ID NO:248);
KLPTVLR (SEQ ID NO:249);
CRFHGNR (SEQ ID NO:250);

YTRDFEA (SEQ ID NO: 251);
SSAAGPR (SEQ ID NO: 252);
SLIQYSR (SEQ ID NO: 253);
DALMWP Xaa (SEQ ID NO: 254);
SS Xaa SLYI (SEQ ID NO: 255);
FNTSTRT (SEQ ID NO: 256);
TVQHVAF (SEQ ID NO: 257);
DYSFPPL (SEQ ID NO: 258);
VGSMESL (SEQ ID NO: 259);
F Xaa PMI Xaa S (SEQ ID NO: 260);
APPRVTM (SEQ ID NO: 261);
IATKTPK (SEQ ID NO: 262);
KPPLFQI (SEQ ID NO: 263);
YHTAHNM (SEQ ID NO: 264);
SYIQATH (SEQ ID NO: 265);
SSFATFL (SEQ ID NO: 266);
TTPPNFA (SEQ ID NO: 267);
ISLDPRM (SEQ ID NO: 268);
SLPLFGA (SEQ ID NO: 269);
NLLKTTL (SEQ ID NO: 270);
DQNLPRR (SEQ ID NO: 271);
SHFEQLL (SEQ ID NO: 272);
TPQLHHG (SEQ ID NO: 273);
APLDRIT (SEQ ID NO: 274);
FAPLIAH (SEQ ID NO: 275);
SWIQTFM (SEQ ID NO: 276);
NTWPHMY (SEQ ID NO: 277);
EPLPTTL (SEQ ID NO: 278);
HGPHLFN (SEQ ID NO: 279);
YLNSTLA (SEQ ID NO: 280);
HLHSPSG (SEQ ID NO: 281);
TLPHRLN (SEQ ID NO: 282);

SSPREVH (SEQ ID NO: 283);
NQVDTAR (SEQ ID NO: 284);
YPTPLLT (SEQ ID NO: 285);
HPAAFPW (SEQ ID NO: 286);
LLPHSSA (SEQ ID NO: 287);
LETYTAS (SEQ ID NO: 288);
KYVPLPP (SEQ ID NO: 289);
APLALHA (SEQ ID NO: 290);
YESLLTK (SEQ ID NO: 291);
SHAASGT (SEQ ID NO: 292);
GLATVKS (SEQ ID NO: 293);
GATSFGL (SEQ ID NO: 294);
KPPGPVS (SEQ ID NO: 295);
TLYVSGN (SEQ ID NO: 296);
HAPFKSQ (SEQ ID NO: 297);
VAFTRLP (SEQ ID NO: 298);
LPTRTPA (SEQ ID NO: 299);
ASFDLLI (SEQ ID NO: 300);
RMNTEPP (SEQ ID NO: 301);
KMTPLTT (SEQ ID NO: 302);
ANATPLL (SEQ ID NO: 303);
TIWPPPV (SEQ ID NO: 304);
QTKVMTT (SEQ ID NO: 305);
NHAVFAS (SEQ ID NO: 306);
LHAA Xaa TS (SEQ ID NO: 307);
TWQPYFH (SEQ ID NO: 308);
APLALHA (SEQ ID NO: 309);
TAHDLTV (SEQ ID NO: 310);
NMTNMLT (SEQ ID NO: 311);
GSGLSQD (SEQ ID NO: 312);
TPIKTIY (SEQ ID NO: 313);
SHLYRSS (SEQ ID NO: 314);

HGQAWQF (SEQ ID NO: 315); and
FHWWW (SEQ ID NO: 317).

[0059] The aforementioned heat shock protein binding domains are merely exemplary of various peptides, among peptide and non-peptide heat shock protein binding molecules, that may be used in the practice of the present invention. In other embodiments, the heat shock protein binding domain may be directed to bind to a different part of the mammalian heat shock protein that those aforementioned, and the heat shock protein-binding domains of the invention are not limited to binding to any particular portion of the heat shock protein molecule. In a non-limiting example, the peptide IFAGIKKKAERADLIAYLKQATAK (Greene et al., 1995, J. Biol. Chem. 270:2967-2973; SEQ ID NO:331) or a heat shock protein-binding fragment of this peptide, is used in any of the conjugates of the invention to facilitate the binding of a pre-selected molecule to a heat shock protein. In addition to the aforementioned peptides that bind to heat shock proteins, the binding may be achieved through the use of an organic molecule or compound with heat shock protein binding activity. For example, suitable molecules include members of the benzoquinone ansamycin antibiotics, such as herbimycin A, geldanamycin, macmimycin I, mimosamycin, and kuwaitimycin (Omura et al., 1979, J. Antibiotics 32:255-261; see also WO9922761, incorporated by reference herein in its entirety), or structurally related compounds, and analogs or derivatives thereof. These molecules may be conjugated though established chemical means to the antigenic domains of the invention, via the peptide linker, to produce hybrid antigens capable of binding to a heat shock protein *in vitro* or *in vivo* and eliciting an immune response to the antigen present therein.

[0060] As described in co-pending and commonly-owned application serial no. 10/776,521, filed February 12, 2004, incorporated herein by reference in its entirety, it has been found that incorporation of a tryptophan residue (Trp, or single amino acid code W) at the C-terminus of the heat shock protein binding domains such as but not limited to those identified as described above, enhances binding to heat shock proteins. Increased binding to heat shock proteins has been found to increase the ability of hybrid antigens to induce an immune response to the antigenic domain of the hybrid antigen, whether administered in a complex with a heat shock protein or when administered alone. Increased immune response is correlated with increased efficacy of treating disease.Other examples of methods for

determining affinity are described in PCT/US96/13363 (WO9706821), which is incorporated herein by reference in its entirety.

[0061] Among the foregoing selection of heat shock protein binding domains, those preferred in the present invention as part of a hybrid antigen comprising an antigenic domain and peptide linker of the invention there between includes the following heat shock protein binding domains:

[0062] Gly Lys Trp Val Tyr Ile Gly Trp (SEQ ID NO:);
Ala Lys Arg Glu Thr Lys Gly Trp (SEQ ID NO:);
Lys Trp Val His Leu Phe Gly Trp (SEQ ID NO:);
Arg Leu Val Leu Val Leu Gly Trp (SEQ ID NO:);
Trp Lys Trp Gly Ile Tyr Gly Trp (SEQ ID NO:);
Ser Ser His Ala Ser Ala Gly Trp (SEQ ID NO:);
Trp Gly Pro Trp Ser Phe Gly Trp (SEQ ID NO:);
Ala Ile Pro Gly Lys Val Gly Trp (SEQ ID NO:);
Arg Val His Asp Pro Ala Gly Trp (SEQ ID NO:);
Arg Ser Val Ser Ser Phe Gly Trp (SEQ ID NO:);
Leu Gly Thr Arg Lys Gly Gly Trp (SEQ ID NO:);
Lys Asp Pro Leu Phe Asn Gly Trp (SEQ ID NO:);
Leu Ser Gln His Thr Asn Gly Trp (SEQ ID NO:);
Asn Arg Leu Leu Leu Thr Gly Trp (SEQ ID NO:);
Tyr Pro Leu Trp Val Ile Gly Trp (SEQ ID NO:);
Leu Leu Ile Ile Asp Arg Gly Trp (SEQ ID NO:);
Arg Val Ile Ser Leu Gln Gly Trp (SEQ ID NO:);
Glu Val Ser Arg Glu Asp Gly Trp (SEQ ID NO:);
Ser Ile Leu Arg Ser Thr Gly Trp (SEQ ID NO:);
Pro Gly Leu Val Trp Leu Gly Trp (SEQ ID NO:);
Val Lys Lys Leu Tyr Ile Gly Trp (SEQ ID NO:);
Asn Asn Arg Leu Leu Asp Gly Trp (SEQ ID NO:);
Ser Lys Gly Arg Trp Gly Gly Trp (SEQ ID NO:);
Ile Arg Pro Ser Gly Ile Gly Trp (SEQ ID NO:);
Ala Ser Leu Cys Pro Thr Gly Trp (SEQ ID NO:);

Asp Val Pro Gly Leu Arg Gly Trp (SEQ ID NO:);
Arg His Arg Glu Val Gln Gly Trp (SEQ ID NO:);
Leu Ala Arg Lys Arg Ser Gly Trp (SEQ ID NO:);
Ser Val Leu Asp His Val Gly Trp (SEQ ID NO:);
Asn Leu Leu Arg Arg Ala Gly Trp (SEQ ID NO:);
Ser Gly Ile Ser Ala Trp Gly Trp (SEQ ID NO:);
Phe Tyr Phe Trp Val Arg Gly Trp (SEQ ID NO:);
Lys Leu Phe Leu Pro Leu Gly Trp (SEQ ID NO:);
Thr Pro Thr Leu Ser Asp Gly Trp (SEQ ID NO:);
Thr His Ser Leu Ile Leu Gly Trp (SEQ ID NO:);
Leu Leu Leu Leu Ser Arg Gly Trp (SEQ ID NO:);
Leu Leu Arg Val Arg Ser Gly Trp (SEQ ID NO:);
Glu Arg Arg ser Arg Gly Gly Trp (SEQ ID NO:);
Arg Met Leu Gln Leu Ala Gly Trp (SEQ ID NO:);
Age Gly Trp Ala Asn Ser Gly Trp (SEQ ID NO:);
Arg Pro Phe Tyr Ser Tyr Gly Trp (SEQ ID NO:);
Ser Ser Ser Trp Asn Ala Gly Trp (SEQ ID NO:);
Leu Gly His Leu Glu Glu Gly Trp (SEQ ID NO:);
Ser Ala Val Thr Asn Thr Gly Trp (SEQ ID NO:);
Phe Tyr Gln Leu Ala Leu Thr (SEQ ID NO:);
Phe Tyr Gln Leu Ala Leu Thr Trp (SEQ ID NO:),
Arg Lys Leu Phe Phe Asn Leu Arg (SEQ ID NO:),
Arg Lys Leu Phe Phe Asn Leu Arg Trp (SEQ ID NO:),
Lys Phe Glu Arg Gln (SEQ ID NO:),
Asn Ile Val Arg Lys Lys Lys (SEQ ID NO:), and
Arg Gly Tyr Val Tyr Gln Gly Leu (SEQ ID NO:).

[0063] Other non-limiting examples of such heat shock protein binding domains with a terminal Trp residue useful for the various aspects of the present invention include:

[0064] Asn Leu Leu Arg Leu Thr Gly Trp (SEQ ID NO:350);
Phe Tyr Gln Leu Ala Leu Tyr Trp (SEQ ID NO:351);
Arg Lys Leu Phe Phe Asn Leu Arg Trp (SEQ ID NO:352);

Gly Lys Trp Val Tyr Ile Gly Trp (SEQ ID NO:295);
Ala Lys Arg Glu Thr Lys Gly Trp (SEQ ID NO:296);
Lys Trp Val His Leu Phe Gly Trp (SEQ ID NO:297);
Arg Leu Val Leu Val Leu Gly Trp (SEQ ID NO:298);
Trp Lys Trp Gly Ile Tyr Gly Trp (SEQ ID NO:299);
Ser Ser His Ala Ser Ala Gly Trp (SEQ ID NO:300);
Trp Gly Pro Trp Ser Phe Gly Trp (SEQ ID NO:301);
Ala Ile Pro Gly Lys Val Gly Trp (SEQ ID NO:302);
Arg Val His Asp Pro Ala Gly Trp (SEQ ID NO:303);
Arg Ser Val Ser Ser Phe Gly Trp (SEQ ID NO:304);
Leu Gly Thr Arg Lys Gly Gly Trp (SEQ ID NO:305);
Lys Asp Pro Leu Phe Asn Gly Trp (SEQ ID NO:306);
Leu Ser Gln His Thr Asn Gly Trp (SEQ ID NO:307);
Asn Arg Leu Leu Leu Thr Gly Trp (SEQ ID NO:308);
Tyr Pro Leu Trp Val Ile Gly Trp (SEQ ID NO:309);
Leu Leu Ile Ile Asp Arg Gly Trp (SEQ ID NO:310);
Arg Val Ile Ser Leu Gln Gly Trp (SEQ ID NO:311);
Glu Val Ser Arg Glu Asp Gly Trp (SEQ ID NO:312);
Ser Ile Leu Arg Ser Thr Gly Trp (SEQ ID NO:313);
Pro Gly Leu Val Trp Leu Gly Trp (SEQ ID NO:314);
Val Lys Lys Leu Tyr Ile Gly Trp (SEQ ID NO:315);
Asn Asn Arg Leu Leu Asp Gly Trp (SEQ ID NO:316);
Ser Lys Gly Arg Trp Gly Gly Trp (SEQ ID NO:317);
Ile Arg Pro Ser Gly Ile Gly Trp (SEQ ID NO:318);
Ala Ser Leu Cys Pro Thr Gly Trp (SEQ ID NO:319);
Asp Val Pro Gly Leu Arg Gly Trp (SEQ ID NO:320);
Arg His Arg Glu Val Gln Gly Trp (SEQ ID NO:321);
Leu Ala Arg Lys Arg Ser Gly Trp (SEQ ID NO:322);
Ser Val Leu Asp His Val Gly Trp (SEQ ID NO:323);
Asn Leu Leu Arg Arg Ala Gly Trp (SEQ ID NO:324);
Ser Gly Ile Ser Ala Trp Gly Trp (SEQ ID NO:325);
Phe Tyr Phe Trp Val Arg Gly Trp (SEQ ID NO:326);

Lys Leu Phe Leu Pro Leu Gly Trp (SEQ ID NO:327);
Thr Pro Thr Leu Ser Asp Gly Trp (SEQ ID NO:328);
Thr His Ser Leu Ile Leu Gly Trp (SEQ ID NO:329);
Leu Leu Leu Leu Ser Arg Gly Trp (SEQ ID NO:330);
Leu Leu Arg Val Arg Ser Gly Trp (SEQ ID NO:331);
Glu Arg Arg ser Arg Gly Gly Trp (SEQ ID NO:332);
Arg Met Leu Gln Leu Ala Gly Trp (SEQ ID NO:333);
Age Gly Trp Ala Asn Ser Gly Trp (SEQ ID NO:334);
Arg Pro Phe Tyr Ser Tyr Gly Trp (SEQ ID NO:335);
Ser Ser Ser Trp Asn Ala Gly Trp (SEQ ID NO:336);
Leu Gly His Leu Glu Glu Gly Trp (SEQ ID NO:337);
Ser Ala Val Thr Asn Thr Gly Trp (SEQ ID NO:338);
Leu Arg Arg Ala Ser Leu Trp (SEQ ID NO:339);
Leu Arg Arg Trp Ser Leu Trp (SEQ ID NO:340);
Lys Trp Val His Leu Phe Trp (SEQ ID NO:341);
Asn Arg Leu Leu Leu Thr Trp (SEQ ID NO:342);
Ala Arg Leu Leu Leu Thr Trp (SEQ ID NO:343);
Asn Ala Leu Leu Leu Thr Trp (SEQ ID NO:344);
Asn Arg Leu Ala Leu Thr Trp (SEQ ID NO:345);
Asn Leu Leu Arg Leu Thr Trp (SEQ ID NO:346);
Asn Arg Leu Trp Leu Thr Trp (SEQ ID NO:347); and
Asn Arg Leu Leu Leu Ala Trp (SEQ ID NO:348).

[0065] Other heat shock protein binding domains useful in the practice of the present invention include Phe Tyr Gln Leu Ala Leu Thr Trp (SEQ ID NO:501), Phe Tyr Gln Leu Ala Leu Thr Trp (SEQ ID NO:502), Arg Lys Leu Phe Phe Asn Leu Arg Trp (SEQ ID NO:503), Arg Lys Leu Phe Phe Asn Leu Arg Trp (SEQ ID NO:504), Lys Phe Glu Arg Gln Trp (SEQ ID NO:505), Asn Ile Val Arg Lys Lys Lys Trp (SEQ ID NO:506), and Arg Gly Tyr Val Tyr Gln Gly Leu Trp (SEQ ID NO:507).

[0066] Moreover, other heat shock protein binding domains include those described in WO9922761, and may have a terminal Trp residue added to achieve the purposes of the present invention. Xaa represents any amino acid.

Tyr Thr Leu Val Gln Pro Leu Trp (SEQ ID NO: 1149);
Thr Pro Asp Ile Thr Pro Lys Trp (SEQ ID NO: 1150);
Thr Tyr Pro Asp Leu Arg Tyr Trp (SEQ ID NO: 1151);
Asp Arg Thr His Ala Thr Ser Trp (SEQ ID NO: 1152);
Met Ser Thr Thr Phe Tyr Ser Trp (SEQ ID NO: 1153);
Tyr Gln His Ala Val Gln Thr Trp (SEQ ID NO: 1154);
Phe Pro Phe Ser Ala Ser Thr Trp (SEQ ID NO: 1155);
Ser Ser Phe Pro Pro Leu Asp Trp (SEQ ID NO: 1156);
Met Ala Pro Ser Pro Pro His Trp (SEQ ID NO: 1157);
Ser Ser Phe Pro Asp Leu Leu Trp (SEQ ID NO: 1158);
His Ser Tyr Asn Arg Leu Pro Trp (SEQ ID NO: 1159);
His Leu Thr His Ser Gln Arg Trp (SEQ ID NO: 1160);
Gln Ala Ala Gln Ser Arg Ser Trp (SEQ ID NO: 1161);
Phe Ala Thr His His Ile Gly Trp (SEQ ID NO: 1162);
Ser Met Pro Glu Pro Leu Ile Trp (SEQ ID NO: 1163);
Ile Pro Arg Tyr His Leu Ile Trp (SEQ ID NO: 1164);
Ser Ala Pro His Met Thr Ser Trp (SEQ ID NO: 1165);
Lys Ala Pro Val Trp Ala Ser Trp (SEQ ID NO: 1166);
Leu Pro His Trp Leu Leu Ile Trp (SEQ ID NO: 1167);
Ala Ser Ala Gly Tyr Gln Ile Trp (SEQ ID NO: 1168);
Val Thr Pro Lys Thr Gly Ser Trp (SEQ ID NO: 1169);
Glu His Pro Met Pro Val Leu Trp (SEQ ID NO: 1170);
Val Ser Ser Phe Val Thr Ser Trp (SEQ ID NO: 1171);
Ser Thr His Phe Thr Trp Pro Trp (SEQ ID NO: 1172);
Gly Gln Trp Trp Ser Pro Asp Trp (SEQ ID NO: 1173);
Gly Pro Pro His Gln Asp Ser Trp (SEQ ID NO: 1174);
Asn Thr Leu Pro Ser Thr Ile Trp (SEQ ID NO: 1175);
His Gln Pro Ser Arg Trp Val Trp (SEQ ID NO: 1176);
Tyr Gly Asn Pro Leu Gln Pro Trp (SEQ ID NO: 1177);
Phe His Trp Trp Trp Gln Pro Trp (SEQ ID NO: 1178);
Ile Thr Leu Lys Tyr Pro Leu Trp (SEQ ID NO: 1179);
Phe His Trp Pro Trp Leu Phe Trp (SEQ ID NO: 1180);

Thr Ala Gln Asp Ser Thr Gly Trp (SEQ ID NO: 1181);
Phe His Trp Trp Trp Gln Pro Trp (SEQ ID NO: 1182);
Phe His Trp Trp Asp Trp Trp Trp (SEQ ID NO: 1183);
Glu Pro Phe Phe Arg Met Gln Trp (SEQ ID NO: 1184);
Thr Trp Trp Leu Asn Tyr Arg Trp (SEQ ID NO: 1185);
Phe His Trp Trp Trp Gln Pro Trp (SEQ ID NO: 1186);
Gln Pro Ser His Leu Arg Trp Trp (SEQ ID NO: 1187);
Ser Pro Ala Ser Pro Val Tyr Trp (SEQ ID NO: 1188);
Phe His Trp Trp Trp Gln Pro Trp (SEQ ID NO: 1189);
His Pro Ser Asn Gln Ala Ser Trp (SEQ ID NO: 1190);
Asn Ser Ala Pro Arg Pro Val Trp (SEQ ID NO: 1191);
Gln Leu Trp Ser Ile Tyr Pro Trp (SEQ ID NO: 1192);
Ser Trp Pro Phe Phe Asp Leu Trp (SEQ ID NO: 1193);
Asp Thr Thr Leu Pro Leu His Trp (SEQ ID NO: 1194);
Trp His Trp Gln Met Leu Trp Trp (SEQ ID NO: 1195);
Asp Ser Phe Arg Thr Pro Val Trp (SEQ ID NO: 1196);
Thr Ser Pro Leu Ser Leu Leu Trp (SEQ ID NO: 1197);
Ala Tyr Asn Tyr Val Ser Asp Trp (SEQ ID NO: 1198);
Arg Pro Leu His Asp Pro Met Trp (SEQ ID NO: 1199);
Trp Pro Ser Thr Thr Leu Phe Trp (SEQ ID NO: 1200);
Ala Thr Leu Glu Pro Val Arg Trp (SEQ ID NO: 1201);
Ser Met Thr Val Leu Arg Pro Trp (SEQ ID NO: 1202);
Gln Ile Gly Ala Pro Ser Trp Trp (SEQ ID NO: 1203);
Ala Pro Asp Leu Tyr Val Pro Trp (SEQ ID NO: 1204);
Arg Met Pro Pro Leu Leu Pro Trp (SEQ ID NO: 1205);
Ala Lys Ala Thr Pro Glu His Trp (SEQ ID NO: 1206);
Thr Pro Pro Leu Arg Ile Asn Trp (SEQ ID NO: 1207);
Leu Pro Ile His Ala Pro His Trp (SEQ ID NO: 1208);
Asp Leu Asn Ala Tyr Thr His Trp (SEQ ID NO: 1209);
Val Thr Leu Pro Asn Phe His Trp (SEQ ID NO: 1210);
Asn Ser Arg Leu Pro Thr Leu Trp (SEQ ID NO: 1211);
Tyr Pro His Pro Ser Arg Ser Trp (SEQ ID NO: 1212);

Gly Thr Ala His Phe Met Tyr Trp (SEQ ID NO: 1213);
Tyr Ser Leu Leu Pro Thr Arg Trp (SEQ ID NO: 1214);
Leu Pro Arg Arg Thr Leu Leu Trp (SEQ ID NO: 1215);
Thr Ser Thr Leu Leu Trp Lys Trp (SEQ ID NO: 1216);
Thr Ser Asp Met Lys Pro His Trp (SEQ ID NO: 1217);
Thr Ser Ser Tyr Leu Ala Leu Trp (SEQ ID NO: 1218);
Asn Leu Tyr Gly Pro His Asp Trp (SEQ ID NO: 1219);
Leu Glu Thr Tyr Thr Ala Ser Trp (SEQ ID NO: 1220);
Ala Tyr Lys Ser Leu Thr Gln Trp (SEQ ID NO: 1221);
Ser Thr Ser Val Tyr Ser Ser Trp (SEQ ID NO: 1222);
Glu Gly Pro Leu Arg Ser Pro Trp (SEQ ID NO: 1223);
Thr Thr Tyr His Ala Leu Gly Trp (SEQ ID NO: 1224);
Val Ser Ile Gly His Pro Ser Trp (SEQ ID NO: 1225);
Thr His Ser His Arg Pro Ser Trp (SEQ ID NO: 1226);
Ile Thr Asn Pro Leu Thr Thr Trp (SEQ ID NO: 1227);
Ser Ile Gln Ala His His Ser Trp (SEQ ID NO: 1228);
Leu Asn Trp Pro Arg Val Leu Trp (SEQ ID NO: 1229);
Tyr Tyr Tyr Ala Pro Pro Pro Trp (SEQ ID NO: 1230);
Ser Leu Trp Thr Arg Leu Pro Trp (SEQ ID NO: 1231);
Asn Val Tyr His Ser Ser Leu Trp (SEQ ID NO: 1232);
Asn Ser Pro His Pro Pro Thr Trp (SEQ ID NO: 1233);
Val Pro Ala Lys Pro Arg His Trp (SEQ ID NO: 1234);
His Asn Leu His Pro Asn Arg Trp (SEQ ID NO: 1235);
Tyr Thr Thr His Arg Trp Leu Trp (SEQ ID NO: 1236);
Ala Val Thr Ala Ala Ile Val Trp (SEQ ID NO: 1237);
Thr Leu Met His Asp Arg Val Trp (SEQ ID NO: 1238);
Thr Pro Leu Lys Val Pro Tyr Trp (SEQ ID NO: 1239);
Phe Thr Asn Gln Gln Tyr His Trp (SEQ ID NO: 1240);
Ser His Val Pro Ser Met Ala Trp (SEQ ID NO: 1241);
His Thr Thr Val Tyr Gly Ala Trp (SEQ ID NO: 1242);
Thr Glu Thr Pro Tyr Pro Thr Trp (SEQ ID NO: 1243);
Leu Thr Thr Pro Phe Ser Ser Trp (SEQ ID NO: 1244);

Gly Val Pro Leu Thr Met Asp Trp (SEQ ID NO: 1245);
Lys Leu Pro Thr Val Leu Arg Trp (SEQ ID NO: 1246);
Cys Arg Phe His Gly Asn Arg Trp (SEQ ID NO: 1247);
Tyr Thr Arg Asp Phe Glu Ala Trp (SEQ ID NO: 1248);
Ser Ser Ala Ala Gly Pro Arg Trp (SEQ ID NO: 1249);
Ser Leu Ile Gln Tyr Ser Arg Trp (SEQ ID NO: 1250);
Asp Ala Leu Met Trp Pro XAA Trp (SEQ ID NO: 1251);
Ser Ser XAA Ser Leu Tyr Ile Trp (SEQ ID NO: 1252);
Phe Asn Thr Ser Thr Arg Thr Trp (SEQ ID NO: 1253);
Thr Val Gln His Val Ala Phe Trp (SEQ ID NO: 1254);
Asp Tyr Ser Phe Pro Pro Leu Trp (SEQ ID NO: 1255);
Val Gly Ser Met Glu Ser Leu Trp (SEQ ID NO: 1256);
Phe XAA Pro Met Ile XAA Ser Trp (SEQ ID NO: 1257);
Ala Pro Pro Arg Val Thr Met Trp (SEQ ID NO: 1258);
Ile Ala Thr Lys Thr Pro Lys Trp (SEQ ID NO: 1259);
Lys Pro Pro Leu Phe Gln Ile Trp (SEQ ID NO: 1260);
Tyr His Thr Ala His Asn Met Trp (SEQ ID NO: 1261);
Ser Tyr Ile Gln Ala Thr His Trp (SEQ ID NO: 1262);
Ser Ser Phe Ala Thr Phe Leu Trp (SEQ ID NO: 1263);
Thr Thr Pro Pro Asn Phe Ala Trp (SEQ ID NO: 1264);
Ile Ser Leu Asp Pro Arg Met Trp (SEQ ID NO: 1265);
Ser Leu Pro Leu Phe Gly Ala Trp (SEQ ID NO: 1266);
Asn Leu Leu Lys Thr Thr Leu Trp (SEQ ID NO: 1267);
Asp Gln Asn Leu Pro Arg Arg Trp (SEQ ID NO: 1268);
Ser His Phe Glu Gln Leu Leu Trp (SEQ ID NO: 1269);
Thr Pro Gln Leu His His Gly Trp (SEQ ID NO: 1270);
Ala Pro Leu Asp Arg Ile Thr Trp (SEQ ID NO: 1271);
Phe Ala Pro Leu Ile Ala His Trp (SEQ ID NO: 1272);
Ser Trp Ile Gln Thr Phe Met Trp (SEQ ID NO: 1273);
Asn Thr Trp Pro His Met Tyr Trp (SEQ ID NO: 1274);
Glu Pro Leu Pro Thr Thr Leu Trp (SEQ ID NO: 1275);
His Gly Pro His Leu Phe Asn Trp (SEQ ID NO: 1276);

Tyr Leu Asn Ser Thr Leu Ala Trp (SEQ ID NO: 1277);
His Leu His Ser Pro Ser Gly Trp (SEQ ID NO: 1278);
Thr Leu Pro His Arg Leu Asn Trp (SEQ ID NO: 1279);
Ser Ser Pro Arg Glu Val His Trp (SEQ ID NO: 1280);
Asn Gln Val Asp Thr Ala Arg Trp (SEQ ID NO: 1281);
Tyr Pro Thr Pro Leu Leu Thr Trp (SEQ ID NO: 1282);
His Pro Ala Ala Phe Pro Trp Trp (SEQ ID NO: 1283);
Leu Leu Pro His Ser Ser Ala Trp (SEQ ID NO: 1284);
Leu Glu Thr Tyr Thr Ala Ser Trp (SEQ ID NO: 1285);
Lys Tyr Val Pro Leu Pro Pro Trp (SEQ ID NO: 1286);
Ala Pro Leu Ala Leu His Ala Trp (SEQ ID NO: 1287);
Tyr Glu Ser Leu Leu Thr Lys Trp (SEQ ID NO: 1288);
Ser His Ala Ala Ser Gly Thr Trp (SEQ ID NO: 1289);
Gly Leu Ala Thr Val Lys Ser Trp (SEQ ID NO: 1290);
Gly Ala Thr Ser Phe Gly Leu Trp (SEQ ID NO: 1291);
Lys Pro Pro Gly Pro Val Ser Trp (SEQ ID NO: 1292);
Thr Leu Tyr Val Ser Gly Asn Trp (SEQ ID NO: 1293);
His Ala Pro Phe Lys Ser Gln Trp (SEQ ID NO: 1294);
Val Ala Phe Thr Arg Leu Pro Trp (SEQ ID NO: 1295);
Leu Pro Thr Arg Thr Pro Ala Trp (SEQ ID NO: 1296);
Ala Ser Phe Asp Leu Leu Ile Trp (SEQ ID NO: 1297);
Arg Met Asn Thr Glu Pro Pro Trp (SEQ ID NO: 1298);
Lys Met Thr Pro Leu Thr Thr Trp (SEQ ID NO: 1299);
Ala Asn Ala Thr Pro Leu Leu Trp (SEQ ID NO: 1300);
Thr Ile Trp Pro Pro Pro Val Trp (SEQ ID NO: 1301);
Gln Thr Lys Val Met Thr Thr Trp (SEQ ID NO: 1302);
Asn His Ala Val Phe Ala Ser Trp (SEQ ID NO: 1303);
Leu His Ala Ala Xaa Thr Ser Trp (SEQ ID NO: 1304);
Thr Trp Gln Pro Tyr Phe His Trp (SEQ ID NO: 1305);
Ala Pro Leu Ala Leu His Ala Trp (SEQ ID NO: 1306);
Thr Ala His Asp Leu Thr Val Trp (SEQ ID NO: 1307);
Asn Met Thr Asn Met Leu Thr Trp (SEQ ID NO: 1308);

Gly Ser Gly Leu Ser Gln Asp Trp (SEQ ID NO: 1309);
Thr Pro Ile Lys Thr Ile Tyr Trp (SEQ ID NO: 1310);
Ser His Leu Tyr Arg Ser Ser Trp (SEQ ID NO: 1311); and
His Gly Gln Ala Trp Gln Phe Trp (SEQ ID NO: 1312).

[0067] Among all of the foregoing heat shock protein binding peptides, the heat shock protein binding domain Asn Leu Leu Arg Leu Thr Gly Trp (SEQ ID NO:350) is most preferred in the hybrid antigens of the invention. However, the aforementioned heat shock protein binding domains are merely exemplary of various moieties, among peptide and non-peptide heat shock protein binding molecules, that may be used in the practice of the present invention.

[0068] The hybrid antigen of the invention incorporates at least one antigenic (immunogenic) domain and at least one one heat shock protein-binding domain, separated by at least one peptide linker as described herein. The hybrid antigen of the invention may be synthesized using chemical peptide synthesis methods or it can be synthesized by expression of a nucleic acid construct containing linked sequences encoding the antigenic and heat shock protein binding domains. One suitable technique utilizes initial separate PCR amplification reactions to produce separate DNA segments encoding the two domains, each with a linker segment attached to one end, followed by fusion of the two amplified products in a further PCR step. This technique is referred to as linker tailing. Suitable restriction sites may also be engineered into regions of interest, after which restriction digestion and ligation is used to produce the desired hybrid antigen-encoding sequence.

[0069] As noted herein, the nucleic acid encoding a hybrid antigen of the invention is also suitable for therapeutic use by administration to the subject, where expression in vivo yields the hybrid antigen with the ability of inducing an immune response.

Heat Shock Proteins

[0070] The term "heat shock protein," as used herein, refers to any protein which exhibits increased expression in a cell when the cell is subjected to a stress. In preferred non-limiting embodiments, the heat shock protein is originally derived from a eukaryotic cell; in more preferred embodiments, the heat shock protein is originally derived from a mammalian cell. For example, but not by way of limitation, heat shock proteins which may

be used according to the invention include BiP (also referred to as grp78), hsp70, hsc70, gp96 (grp94), hsp60, hsp40, and hsp90, and members of the families thereof. Especially preferred heat shock proteins are BiP, gp96, and hsp70, as exemplified below. Most preferred is a member of the hsp70 family. Naturally occurring or recombinantly derived mutants of heat shock proteins may also be used according to the invention. For example, but not by way of limitation, the present invention provides for the use of heat shock proteins mutated so as to facilitate their secretion from the cell (for example having mutation or deletion of an element which facilitates endoplasmic reticulum recapture, such as KDEL or its homologues; such mutants are described in PCT Application No. PCT/US96/13233 (WO 97/06685), which is incorporated herein by reference).

[0071] For embodiments of the invention wherein heat shock protein and hybrid antigen are directly administered to the subject in the form of a protein/peptide complex, the heat shock protein may be prepared, using standard techniques, from natural sources, for example as described in Flynn et al., *Science* 245:385-390 (1989), or using recombinant techniques such as expression of a heat shock encoding vector in a suitable host cell such as a bacterial, yeast or mammalian cell. If pre-loading of the heat shock protein with peptides from the host organism is a concern, the heat shock protein can be incubated with ATP and then repurified. Non-limiting examples of methods for preparing recombinant heat shock proteins are set forth below.

[0072] A nucleic acid encoding a heat shock protein may be operatively linked to elements necessary or desirable for expression and then used to express the desired heat shock protein as either a means to produce heat shock protein for use in a protein vaccine or, alternatively, in a nucleic acid vaccine. Elements necessary or desirable for expression include, but are not limited to, promoter/enhancer elements, transcriptional start and stop sequences, polyadenylation signals, translational start and stop sequences, ribosome binding sites, signal sequences and the like. For example, but not by way of limitation, genes for various heat shock proteins have been cloned and sequenced, including, but not limited to, gp96 (human: Genebank Accession No. X15187; Maki et al., *Proc. Natl. Acad. Sci. U.S.A.* 87:5658-5562 (1990); mouse: Genebank Accession No. M16370; Srivastava et al., *Proc. Natl. Acad. Sci. U.S.A.* 84:3807-3811 (1987)), BiP (mouse: Genebank Accession No. U16277; Haas et al., *Proc. Natl. Acad. Sci. U.S.A.* 85:2250-2254 (1988); human: Genebank

Accession No. M19645; Ting et al., *DNA* 7:275-286 (1988)), hsp70 (mouse: Genebank Accession No. M35021; Hunt et al., *Gene* 87:199-204 (1990); human: Genebank Accession No. M24743; Hunt et al, *Proc. Natl. Acad. Sci. U.S.A.* 82:6455-6489 (1995)), and hsp40 (human: Genebank Accession No. D49547; Ohtsuka K., *Biochem. Biophys. Res. Commun.* 197:235-240 (1993)).

METHODS OF ADMINISTRATION

[0073] The hybrid antigens of the invention or complexes of hybrid antigens and heat shock proteins may be administered to a subject using either a peptide-based, protein-based or nucleic acid vaccine, so as to produce, in the subject, an amount of complex which is effective in inducing a therapeutic immune response in the subject.

[0074] The subject may be a human or nonhuman subject.

[0075] The term "therapeutic immune response," as used herein, refers to an increase in humoral and/or cellular immunity, as measured by standard techniques, which is directed toward the hybrid antigen. Preferably, but not by way of limitation, the induced level of humoral immunity directed toward hybrid antigen is at least four-fold, and preferably at least 16-fold greater than the levels of the humoral immunity directed toward the antigen prior to the administration of the compositions of this invention to the subject. The immune response may also be measured qualitatively, by means of a suitable *in vitro* or *in vivo* assay, wherein an arrest in progression or a remission of neoplastic or infectious disease in the subject is considered to indicate the induction of a therapeutic immune response.

[0076] Specific amounts of heat shock protein/hybrid antigen administered may depend on numerous factors including the immunogenicity of the particular vaccine composition, the immunocompetence of the subject, the size of the subject and the route of administration. Determining a suitable amount of any given composition for administration is a matter of routine screening.

[0077] Furthermore, significant immunological efficacy was identified in studies in which the hybrid antigen was administered alone, i.e., without heat shock protein. While Applicants have no duty to disclose the theory by which the invention operates, and are not bound thereto, the results of these studies suggest that the hybrid antigens, upon injection

into the subject, bind to endogenous heat shock proteins, and thus do not require the concomitant administration of heat shock protein for effectiveness. The present invention extends to such utilities of the hybrid antigens of the invention, and moreover, to concomitant therapies or treatments that increase endogenous heat shock protein levels systemically or at the intended site of administration of the hybrid antigens of the invention. Such concomitant therapies or treatments include but are not limited to local application of heat or local or systemic pharmaceutical agents that increase the expression of heat shock protein in the local tissue. Such agents and methods are known in the art.

[0078] Hybrid antigens that are administered in the absence of co-administration of a heat shock protein (i.e., administered not in a complex with a heat shock protein) that comprise at least one antigenic domain and at least one heat shock protein binding domain comprise one of the peptide linkers mentioned hereinabove.

[0079] In specific non-limiting embodiments of the invention, it may be desirable to include more than one species of heat shock protein, and/or more than one hybrid antigen, in order to optimize the immune response. Such an approach may be particularly advantageous in the treatment of cancer or in the treatment of infections characterized by the rapid development of mutations that result in evasion of the immune response. Moreover, a hybrid antigen of the invention may include more than one immunogenic domain or more than one epitope.

[0080] Compositions comprising hybrid antigen/heat shock protein or hybrid antigen alone as set forth above are referred to herein as "vaccines." The term vaccine is used to indicate that the compositions of the invention may be used to induce a prophylactic or therapeutic immune response. A vaccine of the invention may comprise a hybrid antigen with a single antigenic domain or epitope, or a hybrid antigen with a plurality of antigenic domains or epitopes. Further, a vaccine may comprise an admixture of hybrid antigens with single or pluralities of antigenic domains or epitopes, or any combination of the foregoing. As noted above, the hybrid antigens or admixtures thereof may be complexed with one or more heat shock proteins before administration, or may be administered without heat shock protein.

[0081] A vaccine composition comprising one or more hybrid antigens optionally complexed to one or more heat shock proteins in accordance with the invention may be administered cutaneously, subcutaneously, intradermally, intravenously, intramuscularly, parenterally, intrapulmonarily, intravaginally, intrarectally, nasally or topically. The vaccine composition may be delivered by injection, particle bombardment, orally or by aerosol.

[0082] Incubation of heat shock proteins in solution with the hybrid antigen is sufficient to achieve loading of the antigen onto the heat shock protein in most cases. It may be desirable in some cases, however, to add agents which can assist in the loading of the antigen.

[0083] Incubation with heating of the heat shock protein with the hybrid antigen will in general lead to loading of the antigen onto the heat shock protein. In some cases, however, it may be desirable to add additional agents to assist in the loading. For example, hsp40 can facilitate loading of peptides onto hsp70. Minami et al., *J. Biol. Chem.* 271:19617-19624 (1996). Denaturants such as guanidinium HCl or urea can be employed to partially and reversibly destabilize the heat shock protein to make the peptide binding pocket more accessible to the antigen.

[0084] In particular, a vaccine of the invention comprising a heat shock protein preferably also includes adenosine diphosphate (ADP), to promote the association between the heat shock protein and the heat shock protein binding domain prior to the complex reaching its destination. Other compounds with similar capabilities may used, alone or in combination with ADP.

[0085] Vaccine compositions in accordance with the invention may further include various additional materials, such as a pharmaceutically acceptable carrier. Suitable carriers include any of the standard pharmaceutically accepted carriers, such as phosphate buffered saline solution, water, emulsions such as an oil/water emulsion or a triglyceride emulsion, various types of wetting agents, tablets, coated tablets and capsules. An example of an acceptable triglyceride emulsion useful in intravenous and intraperitoneal administration of the compounds is the triglyceride emulsion commercially known as Intralipid®. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin,

stearic acid, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients.

[0086] The vaccine composition of the invention may also include suitable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions may be in the form of liquid or lyophilized or otherwise dried formulations and may include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), solubilizing agents (e.g. glycerol, polyethylene glycerol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (e.g., lactose, mannitol), covalent attachment of polymers such as polyethylene glycol to the protein, complexing with metal ions, or incorporation of the material into or onto particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, hydrogels, etc. or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts. Such compositions will influence the physical state, solubility, stability, rate of *in vivo* release, and rate of *in vivo* clearance. The choice of compositions will depend on the physical and chemical properties of the vaccine. For example, a product derived from a membrane-bound form of a protein may require a formulation containing detergent. Controlled or sustained release compositions include formulation in lipophilic depots (e.g. fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (e.g. poloxamers or poloxamines) and coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors. Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including intramuscular, parenteral, pulmonary, nasal and oral.

[0087] As an alternative to direct administration of the hybrid antigen optionally complexed with heat shock protein, one or more polynucleotide constructs may be administered which encode the hybrid antigen, optionally with heat shock protein, in expressible form. The expressible polynucleotide constructs are introduced into cells in the subject using *ex vivo* or *in vivo* methods. Suitable methods include injection directly into

tissue and tumors, transfecting using liposomes (Fraley et al., *Nature* 370:111-117 (1980)), receptor-mediated endocytosis (Zatloukal et al., *Ann. NY Acad. Sci.* 660:136-153 (1992)), particle bombardment-mediated gene transfer (Eisenbraun et al., *DNA & Cell Biol.* 12:792-797 (1993)) and transfection using peptide presenting bacteriophage (Barry et al, *Nature Medicine* 2:299-305 (1996). The polynucleotide vaccine may also be introduced into suitable cells *in vitro* which are then introduced into the subject.

[0088] To construct an expressible polynucleotide, a region encoding the heat shock protein and/or hybrid antigen is prepared as discussed above and inserted into a mammalian expression vector operatively linked to a suitable promoter such as the SV40 promoter, the cytomegalovirus (CMV) promoter or the Rous sarcoma virus (RSV) promoter. The resulting construct may then be used as a vaccine for genetic immunization. The nucleic acid polymer(s) could also be cloned into a viral vector. Suitable vectors include but are not limited to retroviral vectors, adenovirus vectors, vaccinia virus vectors, pox virus vectors and adenovirus-associated vectors. Specific vectors which are suitable for use in the present invention are pCDNA3 (InVitrogen), plasmid AH5 (which contains the SV40 origin and the adenovirus major late promoter), pRC/CMV (InVitrogen), pCMU II (Paabo et al., *EMBO J.* 5:1921-1927 (1986)), pZip-Neo SV (Cepko et al., *Cell* 37:1053-1062 (1984)) and pSRα (DNAX, Palo Alto, CA).

[0089] Various methods for preparation of heat shock proteins and hybrid antigens are disclosed in WO9706821 and WO9922761, which are incorporated herein by reference in their entireties.

[0090] In the following examples, and throughout the application amino acids may be represented using their single-letter codes, as follows:

[0091] A alanine

[0092] C cysteine

[0093] D aspartic acid

[0094] E glutamic acid

[0095] F phenylalanine

[0096] G glycine

[0097] H histidine

[0098] I isoleucine

[0099] K lysine

[00100] L leucine

[00101] M methionine

[00102] N asparagine

[00103] P proline

[00104] Q glutamine

[00105] R arginine

[00106] S serine

[00107] T threonine

[00108] V valine

[00109] W tryptophan

[00110] Y tyrosine

[00111] The present invention may be better understood by reference to the following non-limiting Examples, which are provided as exemplary of the invention. The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

EXAMPLE 1

[0100] A variety of hybrid antigens were prepared, each comprising a heat shock protein binding domain and a cancer antigen epitope or the model Class I H2-K^b epitope from ovalbumin, SIINFEKL. A peptide linker was included between the two domains. The heat shock protein binding domains used in these experiments were among the following: HWDFAWPW, NLLRLTGW, FYQLALTW and RKLFFNLRW. Linkers were among those desceibed hereinabove.

[0101] The cancer and model epitopes were among the following:

Source Protein	Source Tumor	Amino Acids	Trivial Name (Amino acid sequence)
Prostate Specific Membrane Antigen	Prostate cancer	771-779	PSMA P2 (ALFDIESKV)
Gp100	Melanoma	209-217	IMD (210M) (IMDQVPFSV)
Tyrosinase	Melanoma	368-376	YMD (370D) (YMDGTMSQV)
Human Papillomavirus (HPV) Strain 16 E7	Cervical cancer	86-93	HPV16 E7 86-93 (TLGIVCPI)
HPV Strain 16 E7	Cervical cancer	11-20	HPV16 E711-20 (YMLDLQPETT)
Ovalbumin	Model Tumor Antigen	257-264	Ova (SIINFEKL)

Using standard solid phase peptide synthesis using F-moc chemistry, hybrid antigens comprising a heat shock protein binding domain, a cancer epitope, and a linker there between, were synthesized, in various orientations.

EXAMPLE 2

[0102] Binding affinities between recombinant human or murine heat shock protein 70 (hsp70) and the various heat shock protein binding domains and antigenic peptides mentioned above, as well as between the hybrid antigens comprising an antigenic peptide and a heat shock protein binding domain described above, were determined by a binding inhibition assays (Hill plots) relative to the binding affinity of a reference, labeled hybrid antigen (tritiated or fluoresceinated ALFDIESKVGSGHWDFAWPW) to hsp70 as determined by Scatchard analysis (Kds of 22.64 μM and 10.75 μM, respectively). Binding studies were performed in 39% PBS; 20 mM THAM, pH 8; 37 mM NaCl, 5 mM $MgCl_2$; and 1 mM ADP.

EXAMPLE 3

For immunological studies in mice, a murine MHC H2-K(b) epitope from ovalbumin, SIINFEKL (amino acids 257-264), and a H2-K(b) peptide from the nucleoprotein of vesicular stomatitis virus (VSV), RGYVYQGL (amino acids 52-59) were used for the

preparation of hybrid antigens. The following table sets forth the sequences and the affinities for hsp70 of the epitopes alone and in hybrid antigens.

Mouse Epitope	Epitope alone		Hybrid antigen comprising epitope	
	Epitope sequence	**Affinity for hsp70 (μM)**	**Hybrid antigen sequence**	**Affinity for hsp70 (μM)**
Ovalbumin: amino acids 257-264	SIINFEKL	235	NLLRLTGWGSGSIINFEKL	1.6
			NLLRLTGWFFRKSIINFEKL	2.2
			NLLRLTGWRKSIINFEKL	0.8
VSV nucleo-protein: amino acids 52-59	RGYVYQGL	82	NLLRLTGWGSGRGYVYQGL	1.4
			NLLRLTGWFFRKRGYVYQGL	1.0
			NLLRLTGWRKRGYVYQGL	0.6

EXAMPLE 4

[0103] Mice were immunized s.c. at the base of the tail with hsp70 alone, hsp70 complexed with SIINFEKL, and hybrid SIINFEKL peptide with or without HSP70. The doses were adjusted such that each immunization contained the same amount of SIINFEKL, except for hsp70 alone. Seven days later, spleens were harvested and enriched for CD8+ T cells, which were put into an *ex vivo* IFN-γ ELISPOT assay. Responses after pulsing with SIINFEKL ("SIINFEKL") were recorded in the following table, which includes the doses, and the number of spots (mean ± standard error) per 4 x 10^5 CD8 T cells, of ≥ four experiments with at least three mice per group. Controls included medium alone ("medium control"), unpulsed T cells ("unpulsed control"), T cells pulsed with a non-immunized peptide derived from VSV, RGYVYQGL ("VSV control"), and exposure to concanavalin A as a positive control ("Con A positive control").

In the same experiment, a ^{51}Cr-release assay as described above was done using SIINFEKL-pulsed target cells. At an effector to target cell ratio of 200:1, the percent killing results obtained are shown in the far right column of the following table.

(200-10)

Immunogen	Number of Spots per 400,000 cells					CTL assay: % killing at 200:1 E/T
	SIINFEKL	Medium control	Unpulsed control	VSV control	Con A positive control	
4.4 µg Hsp70	**0.00 ± 0.00**	1.50±2.12	0.67±0.58	0.33±0.58	834±28.3	**0%**
4.4 µg Hsp70 + 0.9 µg SIINFEKL	**33.7 ± 7.09**	0.00±0.00	0.33±0.58	0.00±0.00	1000±33.7	**19%**
4.4 µg Hsp70 + 2.0 µg NLLRLTGWGSGSIINFEKL	**80.0 ± 17.0**	0.00±0.00	1.50±0.71	1.50±0.71	1170±56.5	**38%**
4.4 µg Hsp70 + 2.4 µg NLLRLTGWFFRKSIINFEKL	**222 ± 17.7**	0.00±0.00	0.67±0.58	1.33±1.53	1010±56.5	**52%**

EXAMPLE 5

[0104] An experiment similar to that described above was carried out, which also included hybrid antigen without hsp70.

(200-11)

Immunogen	Number of Spots per 4 x 10^5 CD8 T cells				
	SIINFEKL	**Medium control**	**Unpulsed control**	**VSV control**	**Con A Positive control**
4.4 μg Hsp70	**0.33 ± 0.58**	1.00 ± 1.73	1.67 ± 1.15	4.00 ±1.00	965 ± 62.6
4.4 μg Hsp70 + 0.9 μg SIINFEKL	**1.67 ± 0.58**	1.00 ± 1.00	2.00 ± 0.00	2.67 ± 2.08	591 ± 48.1
4.4 μg Hsp70 + 2.0 μg NLLRLTGWGSGSIINFEKL	**12.0 ± 5.2**	2.67 ± 0.58	1.67 ± 1.15	2.00 ± 2.65	748 ± 58.6
4.4 μg Hsp70 + 2.4 μg NLLRLTGWFFRKSIINFEKL	**770 ± 80.6**	3.33 ± 1.53	3.67 ± 1.53	4.33 ± 1.53	742 ± 72.6
2.4 μg NLLRLTGWFFRKSIINFEKL (no hsp70)	**151 ± 20.7**	1.00 ± 1.00	1.67 ± 0.58	0.00 ± 0.00	459 ± 149

EXAMPLE 6

A further experiment was carried out similar to that described above.

(200-12)

Immunogen	Number of spots per 300,000 CD8 T cells					CTL assay: % killing at 200:1 E/T
	SIINFEKL	Medium control	Unpulsed control	VSV control	Con A positive control	
4.4 μg Hsp70	**0.67 ± 0.58**	0.00 ± 0.00	0.50 ± 0.71	1.00 ± 1.41	552 ± 24.0	**8.45 ± 41.3**
4.4 μg Hsp70 + 0.9 μg SIINFEKL	**3.33 ± 2.52**	0.00 ± 0.00	0.33 ± 0.58	0.33 ± 0.58	450 ± 69.0	**43.0 ± 21.2**
4.4 μg Hsp70 + 2.00 μg NLLRLTGWGSG-SIINFEKL	**134 ± 4.16**	1.33 ± 1.53	0.67 ± 1.15	1.00 ± 1.00	865 ± 93.0	**31.9 ± 5.41**
4.4 μg Hsp70 + 2.4 μg NLLRLTGWFFRK-SIINFEKL	**680 ± 23.0**	0.00 ± 0.00	0.00 ± 0.00	1.67 ± 0.58	801 ± 56.6	**84.6 ± 1.70**
2.4 μg NLLRLTGWFFRK-SIINFEKL	**211 ± 17.0**	0.00 ± 0.00	0.50 ± 0.71	1.00 ± 0.00	688 ± 41.7	**9.91 ± 5.57**

EXAMPLE 7

[0105] As in the prior in vivo experiments, B6 mice were immunized s.c. to evaluate complexes of hsp70 with hybrid antigens made using other short peptide linkers, including (using one-letter amino-acid codes) FFRK, RK, AKVL, QLK and FR, and at different doses. An *ex vivo* IFN-γ ELISPOT assay was performed as described above. The results including the control values are as follows.

(200-13)

Immunogen	Number of Spots per 300,000 cells			
	SIINFEKL	**Medium control**	**Unpulsed control**	**VSV control**
4.4 μg Hsp70 + 2.4μg NLLRLTGWFFRKSIINFEKL	**114 ± 21**	1.0 ± 1.2	1.0 ± 0	0.67 ± 0.41
4.4 μg Hsp70 + 2.4μg NLLRLTGWRKSIINFEKL	**70 ± 8.5**	1.3 ± 1.1	0.67 ± 0.82	2.7 ± 1.1
0.9 μg Hsp70 + 0.48μg NLLRLTGWFFRKSIINFEKL	**98 ± 0.41**	.67 ± 0.82	1.3 ± 1.1	4.3 ± 2.3
0.9 μg Hsp70 + 0.48μg NLLRLTGWRKSIINFEKL	**29 ± 2.2**	0 ± 0	1 ± 0	0 ± 0
2.4μg NLLRLTGWFFRKSIINFEKL	**11 ± 1.8**	0.67 ± 0.82	0 ± 0	0.67 ± 0.82

200-21

Immunogen	Number of Spots per 400,000 cells			
	SIINFEKL	**Medium control**	**Unpulsed control**	**VSV control**
4.4 μg Hsp70 + 2.4μg NLLRLTGWFFRKSIINFEKL	**124 ± 8.8**	0.33 ± 0.41	0.67 ± 0.82	2.67 ± 2.68
4.4 μg Hsp70 + 2.4μg NLLRLTGWAKVLSIINFEKL	**95 ± 12**	1.33 ± 0.82	1.0 ± 1.2	0.67 ± 0.41

Immunogen	Number of Spots per 400,000 cells			
	SIINFEKL	**Medium control**	**Unpulsed control**	**VSV control**
4.4 μg Hsp70 + 2.4 μg NLLRLTGWFFRKSIINFEKL	**318 ± 17**	0.67 ± 0.51	0.67 ± 0.58	0.67 ± 0.58
4.4 μg Hsp70 + 2.4 μg NLLRLTGWQLKSIINFEKL	**174 ± 18**	0.0 ± 0.0	0.0 ± 0.0	3.7 ± 2.5
4.4 μg Hsp70 + 2.4 μg NLLRLTGWFRSIINFEKL	**53 ± 2.9**	0.0 ± 0.0	0.67 ± 0.58	1.0 ± 1.0
2.4 μg NLLRLTGWFRSIINFEKL	**31 ± 5.7**	1.0 ± 1.7	0.0 ± 0.0	0.67 ± 0.58

EXAMPLE 8

Similar in vivo studies in B6 mice as those described above were performed using formulations without added hsp70. The results are as follows.

(200-17)

Immunogen	Number of Spots per 400,000 cells				
	SIINFEKL	**Medium control**	**Unpulsed control**	**VSV control**	**Con A positive control**
10 μg SIINFEKL	**2.33 ± 0.41**	0.33 ± 0.41	1.33 ± 0.82	1.7 ± 0.41	928 ± 72
0.5 μg NLLRLTGWFFRKSIINFEKL	**22 ± 7.2**	1.33 ± 0.41	1.67 ± 1.1	1.0 ± 0.71	906 ± 17
2.5 μg NLLRLTGWFFRKSIINFEKL	**28 ± 2.7**	1.0 ± 1.7	0.33 ± 0.41	2.0 ± 1.2	930 ± 23
25 μg NLLRLTGWFFRKSIINFEKL	**46 ± 4.3**	2.0 ± 0.41	1.33 ± 1.1	3.0 ± 0.71	1007 ± 17

EXAMPLE 9

Similar in vivo studies in B6 mice as those described above were performed using formulations with or without hsp70. In addition, one study was carried out in which hybrid antigen was co-administered with free heat shock protein-binding domain peptide (NLLRLTGW). The results are as follows.

(VSV-72-02)

Immunogen	Number of spots per 400,000 cells					
	SIINFEKL	**Medium control**	**Unpulsed control**	**VSV control**	**Con A positive control**	**CTL % killing at 200:1 E/T**
4μg Hsp70 + 2.0μg NLLRLTGWFFRKSIINFEKL	**48 ± 11**	0.0 ± 1.0	0.0 ± 1.0	4.0 ± 2.0	588 ± 151	32%
2.0μg NLLRLTGWRKSIINFEKL	**24 ± 1**	1.0 ± 1.0	1.0 ± 1.0	5.0 ± 3.0	842 ± 73	24%
2.0 μg NLLRLTGWFFRKSIINFEKL + 50-fold excess NLLRLTGW	**2.0 ± 1.0**	1.0 ± 1.0	0.0 ± 1.0	1.0 ± 1.0	422 ± 54	18%
SIINFEKL	**1.0 ± 1.0**	0.0 ± 0.0	0.0 ± 1.0	1.0 ± 1.0	478 ± 67	6%

EXAMPLE 10

The VSV epitope used as a control in many of the foregoing experiments, RGYVYQGL, was used as the epitope in preparing further hybrid antigens of the invention, and evaluated for induction of an immune response in similar experiments as described above.

(VSV-72-02)

Immunogen	Number of Spots per 400,000 cells			
	SIINFEKL	Medium control	Unpulsed control	VSV (RGYVYQGL)
4μg HSP plus 2μg NLLRLTGWFFRKSIINFEKL	**48 ± 11**	1.0 ± 1.0	0.0 ± 1.0	4.0 ± 2.0
4μg HSP plus 2μg NLLRLTGWFFRKRGYVYQGL	**1.0 ± 1.0**	1.0 ± 1.0	4.0 ± 2.0	20 ± 1.0
4μg HSP plus 6μg NLLRLTGWFFRKRGYVYQGL	**6.0 ± 3.0**	2.0 ± 2.0	12 ± 3.0	104 ± 13

EXAMPLE 11

In order to evaluate the efficacy of the aforementioned hybrid antigens and complexes with hsp70 on the treatment of disease, a model was utilized in which 20,000 E7 tumor cells modified to express ovalbumin (designated E.G7) were subcutaneously implanted in B6 mice. Ten mice were used per treatment group. This model is described, for example, in Moroi et al., 2000, *Proc. Nat. Acad. Sci. USA* **97**:3485-3490. The results in number of mice with tumors over time are shown in **Figure 1**. After 31 days, none of 10 mice immunized with hsp70:NLLRLTGWFFRKSIINFEKL developed tumors, nor did mice immunized with SIINFEKL emulsified in Titermax adjuvant. Three of 10 mice vaccinated with NLLRLTGWFFRKSIINFEKL alone (no hsp70) had tumors. Five of 10 mice vaccinated with hsp70: SIINFEKL had tumors, and 9 of 10 mice immunized with Titermax and buffer alone had tumors.

EXAMPLE 12

The in-vitro antigen presentation assay described above was utilized further in order to evaluate the formulations of the invention. To demonstrate the requirement of the hybrid antigens of the invention for hsp70, whether supplied in the formulation or endogenously

available, for entry of the hybrid antigen and more specifically its antigen into the antigen presentation pathway, the assay was performed with the following formulations, with the results indicated.

(200-MF-41)

Formulation	Pg/ml IL-2 produced by B3Z cells
0.5 ng SIINFEKL	**2690 ± 369**
5 ng NLLRLTGWFFRKSIINFEKL	**46 ± 11**
5 ng NLLRLTGWFFRKSIINFEKL plus 1.4 ug hsp70	**3920 ± 344**
1.4 ug Hsp70	**0.0 ± 0.0**

EXAMPLE 13

[0106] The HHD II mouse model bearing a human HLA-A2 complex described by Firat et al., 1999, "H-2 class I knockout, HLA-A2.1-transgenic mice: a versatile animal model for preclinical evaluation of antitumor immunotherapeutic strategies," *Eur J Immunol.* **29**:3112-21, was used in the following experiments to evaluate human HLA-A2 epitopes in hybrid antigens of the invention. The "IMD" peptide epitope IMDQVPFSV from the human melanoma antigen gp100 was evaluated in a hybrid antigen of the invention at low and high dose in the HHD II model. Similar methods to those described above were used for the ELISPOT assay, with test peptides being the IMD peptide and, as a control, a peptide from the melanoma antigen tyrosinase, YMDGTMSQV ("YMD"). The results are shown on the following table.

(HHD II 200-72-02)

Immunogen	Number of Spots per 400,000 cells			
	IMD	**Medium control**	**Unpulsed control**	**YMD control**
4μg hsp70 and 5μg NLLRLTGWFFRKIMDQVPFSV	**139 ± 11**	0.67 ± 0.58	1.0 ± 1.0	3.7 ± 0.58
4μg hsp70 and 10μg NLLRLTGWFFRKIMDQVPFSV	**217 ± 3.2**	0.67 ± 0.58	4.0 ± 6.0	2.7 ± 1.5
2μg NLLRLTGWFFRKIMDQVPFSV	**27 ± 5.1**	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 2.0

A similar experiment in HHD II mice carried out using YMD as the epitope in the hybrid antigen, in a complex with hsp70, as follows.

(200-72-01)

Immunogen	Number of Spots per 400,000 cells			
	YMD	**Medium control**	**Unpulsed control**	**IMD control**
4μg hsp70 and 5μg NLLRLTGWFFRKYMDGTMSQV	**33 ± 7.8**	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 1.4
4μg hsp70 and 10μg NLLRLTGWFFRKYMDGTMSQV	**323 ± 44**	0.0 ± 0.0	1.5 ± 0.71	1.5 ± 0.71

EXAMPLE 14

An epitope from Sendai virus (SdV), FAPGNYPAL, was evaluated in hybrid antigens of the invention in B6 mice, similar to the above. The results are as follows.

(200-18)

Immunogen	Number of Spots per 400,000 cells		
	SdV	Medium control	SIINFEKL control
4μg hsp70 and 2μg NLLRLTGWFFRKSIINFEKL	1.3 ± 1.2	1.0 ± 1.0	**197 ± 27**
2μg NLLRLTGWFFRKRGYVYQGL	0.33 ± 0.58	0.0 ± 0.0	**87 ± 20**
4μg hsp70 and 2μg NLLRLTGWFFRKFAPGNYPAL	**38 ± 17**	0.33 ± 0.58	1.0 ± 1.0
13μg hsp70 and 7μg NLLRLTGWFFRKFAPGNYPAL	**169 ± 32**	4.3 ± 1.5	7.0 ± 3.5

EXAMPLE 15

In-vivo experiments on co-administration of two hybrid antigens of the invention with hsp70 to B6 mice was performed. Hybrid antigens containing SIINFEKL from ovalbumin and RGYVYQGL from VSV were admixed and immunized with hsp70. The results are as follow.

(OVA-VSV-72-01)

Immunogen	Number of Spots per 400,000 cells			
	VSV	Medium control	Unpulsed control	OVA
2μg hsp70 2μg NLLRLTGWFFRKSIINFEKL 2μg NLLRLTGWFFRKRGYVYQGL	**77 ± 19**	2.0 ± 1.0	2.0 ± 1.0	**366 ± 19**
2μg hsp70 6μg NLLRLTGWFFRKSIINFEKL 6μg NLLRLTGWFFRKRGYVYQGL	**185 ± 9**	1.0 ± 1.0	4.0 ± 2.0	**349 ± 10**

EXAMPLE 16

As noted above, in one aspect of the invention, formulations containing a plurality of hybrid antigens comprising different antigenic epitopes may be formulated with one or more heat shock proteins for immunization in humans in order to elicit an effective immune response to treat or prevent a disease. For example, for treating human melanoma, a formulation comprising 8 different melanoma epitopes may be prepared as hybrid antigens, and formulated, for example, with hsp70. In this particular formulation, the heat shock protein binding domain NLLRLTGW at the N-terminus is used for all epitopes, linked to the epitope at the C-terminus using the peptide linker FFRK. Other binding domains and linkers are embraced herein. This particular formulation is useful for treating patients with the HLA-A2 haplotype. A formulation comprises the following hybrid antigens with hsp70:

Source and amino acid sequence of antigen	Hybrid antigen sequence
gp 100: amino acids 209-217 (modified 210M)	NLLRLTGWFFRKIMDQVPFSV
tyrosinase: amino acids 368-376 (modified 370 D)	NLLRLTGWFFRKYMDGTMSQV
Melan-A: amino acids 26-35 (modified 27L)	NLLRLTGWFFRKELAGIGILTV
NY-ESO-1: amino acids 157-165 (modified 165V)	NLLRLTGWFFRKSLLMWITQV
TRP-2: amino acids 180-188	NLLRLTGWFFRKSVYDFFVWL
MAGE-10: amino acids 254-262	GLYDGMEHLGSGNLLRLTGW
gp100: amino acids 280-288 (288V)	YLEPGPVTVGSGNLLRLTGW
SSX-2: amino acids 41-49	KASEKIFYVGSGNLLRLTGW

In one embodiment, approximately equal amounts of the foregoing 8 hybrid antigens may be complexed with hsp70, and administered in saline. In another embodiment, a formulation comprises the first five hybrid antigens listed. The aforementioned formulations containing heat shock protein in saline optionally may contain ADP to stabilize the complexes, as well as other components, such as excipients, diluents and carriers, as mentioned above. In another embodiment, an admixture of the foregoing 8 hybrid antigens, or the first 5 listed, is formulated in saline for administration without a heat shock protein.

EXAMPLE 17

Prime-boost protocols were valuated in this experiment. Using the NLLRLTGWFFRKSIINFEKL hybrid antigen, or without co-administered hsp70, the following 5 protocols were followed: 1) administer at day 0, analyze at day 7; 2) administer at days 0 and 7, analyze at day 21; 3) administer at day 0, analyze at day 21; 4) administer at days 0 and 14, analyze at day 28; and 5) administer at day 0 and analyze at day 28. The results in number of spots per 400,000 cells, were as follows.

(200-28-72-01a, -01b, -01c)

Protocol day(s) immunized	**0**	**0, 7**	**0**	**0, 14**	**0**
Protocol day analyzed	**7**	**21**	**21**	**28**	**28**
SIINFEKL	3.0 ± 2.0	2.0 ± 1.0	0.0 ± 1.0	1.0 ± 1.0	1.0 ± 1.0
2μg hsp70, 4μg SIINFEKL	3.0 ± 1.0	6.0 ± 1.0	22 ± 8.0	3.0 ± 2.0	20 ± 5.0
2μg NLLRLTGWFFRKSIINFEKL	72 ± 5.0	24 ± 6.0	42 ± 7.0	25 ± 9.0	82 ± 11
2μg hsp70 and 4μg NLLRLTGWFFRKSIINFEKL	99 ± 12	98 ± 11	141 ± 14	398 ± 18	27 ± 2.0
2μg hsp70	5.0 ± 6.0	3.0 ± 2.0	3.0 ± 0.0	1.0 ± 1.0	4.0 ± 3.0

EXAMPLE 18

Further experiments were performed with mixtures of hybrid antigens to demonstrate eliciting of an immune response to the component antigens, as above. In this experiment, hybrid antigens containing SIINFEKL and the VSV peptide RGYVYQGL were used.

(VSV/OVA-72-02)

Immunogen	Number of Spots per 300,000 cells			
	SIINFEKL	**Medium control**	**Unpulsed control**	**VSV (RGYVYQGL)**
3.7 μg hsp70 2μg NLLRLTGWFFRKSIINFEKL	**238 ± 27**	0.0 ± 0.0	1.0 ± 1.0	**5.0 ± 2.0**
11.2 μg hsp70 6μg NLLRLTGWFFRKSIINFEKL	**330 ± 45**	1.0 ± 1.0	0.0 ± 0.0	**4.0 ± 1.0**
3.7 μg hsp70 2μg NLLRLTGWFFRKRGYVYQGL	**1.0 ± 1.0**	1.0 ± 1.0	0.0 ± 0.0	**61 ± 11**
11.2 μg hsp70 6μg NLLRLTGWFFRKRGYVYQGL	**2.0 ± 2.0**	2.0 ± 1.0	2.0 ± 0.0	**147 ± 20**
3.7 μg hsp70 2μg NLLRLTGWFFRKSIINFEKL 2μg NLLRLTGWFFRKRGYVYQGL	**179 ± 4.0**	2.0 ± 2.0	1.0 ± 1.0	**165 ± 11**
11.2 μg hsp70 6μg NLLRLTGWFFRKSIINFEKL 6μg NLLRLTGWFFRKRGYVYQGL	**310 ± 13**	1.0 ± 1.0	1.0 ± 1.0	**242 ± 52**

EXAMPLE 19

The binding affinity for hybrid antigens comprising heat shock protein binding domain NLLRLTGW, antigenic domain SIINFEKL (from ovalbumin) or RGYVYQGL (from VSV protein) and various linkers set forth in Example 32 were carried out as described in Example 17. The antigenic domains alone had a Kd for hsp70 binding of 235 μM and 82 μM, respectively. The results are shown below.

Hybrid antigen	Kd for binding to HSP70
NLLRLTGWGSGSIINFEKL	1.6 μM
NLLRLTGWFFRKSIINFEKL	2.2 μM
NLLRLTGWRKSIINFEKL	0.8 μM
NLLRLTGWAKVLSIINFEKL	2.0 μM
NLLRLTGWQLKSIINFEKL	0.4 μM
NLLRLTGWFRSIINFEKL	1.5 μM
NLLRLTGWGSGRGYVYQGL	1.4 μM
NLLRLTGWFFRKRGYVYQGL	1.0 μM
NLLRLTGWRKRGYVYQGL	0.6 μM

EXAMPLE 20

Further studies were carried out to evaluate the immunogenicity of hybrid antigens when administered alone to B6 mice, without co-administration of hsp70. The methods for evaluation using IFN-γ ELISPOT are as described above.

(Control 200-24 and 200-30)

Immunogen	Number of Spots per 300,000 cells			
	SIINFEKL	SWDFITV	Medium	Unpulsed Splenocytes
25 μg NLLRLTGWFFRKSIINFEKL	109 ± 14	NT	0 ± 0	3.0 ± 2.0
24.9 μg NLLRLTGWFFRKSSWDFITV	NT	26 ± 5	0.67 ± 0.58	0.33 ± 0.58
2.1 μg NLLRLTGWFRSIINFEKL	12 ± 2	NT	0.67 ± 0.58	0.67 ± 0.58

NT not tested

EXAMPLE 21

Hybrid antigens were prepared comprising two antigens, separated by a linker as described above, such that the hybrid antigen has the following general structure:

(Heat shock protein binding domain) – (linker) – (Antigen 1) – (linker) – (Antigen 2).

While in this example the heat shock protein binding domain is at the N-terminal portion of the hybrid antigen, this is not necessarily the case and hybrid antigens with the heat shock protein binding domain at the C-terminus, or in-between the two antigenic domains, is embraced by the present invention. Furthermore, although in the examples below the same linker peptide is used between the antigenic domains and between the antigenic domain proximal to the heat shock protein binding domain, this is not necessarily the case and different linker peptides may be used. Moreover, the presence of the linker in one or both positions is optional. And furthermore, three or more antigenic peptides may be used. For simplicity, such hybrid antigens with two or more antigenic domains is termed a tandem hybrid antigen. Such tandem hybrid antigen compositions, complexes of one or more tandem hybrid antigens and a heat shock protein, and methods of eliciting an immune response or preventing or treating a disease by administering one or more tandem hybrid antigens or complexes of at least one heat shock protein and at least one tandem hybrid antigen are fully embraced herein.

The following experiments compare the immunogenicity of the admixture of two hybrid antigens and a tandem hybrid antigen comprising the same antigens, and a dose response study. In one experiment, a peptide comprising two linkers and epitopes but no heat shock protein binding domain was included.

(Control-200-72-01)

Immunogen	Number of Spots per 300,000 cells			
	SIINFEKL	Medium control	Unpulsed control	RGYVYQGL
19.2 µg NLLRLTGWFFRKSIINFEKLFFRKRGYVYGL	**390 ± 56**	1.7 ± 1.1	3.0 ± 1.9	**146 ± 13**
19.2 µg NLLRLTGWFFRKRGYVYQGLFFRKSIINFEKL	**180 ± 11**	1.3 ± 1.1	2.7 ± 1.1	**321 ±5.8**

(S200-72-02)

Immunogen	Number of Spots per 300,000 cells		
	SIINFEKL	Medium control	RGYVYQGL
7.3 µg FFRKSIINFEKLFFRKRGYVYQGL	**8.3 ± 1.1**	**1.7 ± 0.4**	**31 ± 5.5**
9.6 µg NLLRLTGWFFRKSIINFEKLFFRKRGYVYQGL	**713 ± 13**	**9.0 ± 1.2**	**207 ± 8.2**
9.6 µg NLLRLTGWFFRKRGYVYQGLFFRKSIINFEKL	**69 ± 12**	**0.7 ± 0.4**	**460 ± 14**

(S200-72-12)

Immunogen	Number of Spots per 300,000 cells		
	SIINFEKL	Medium control	RGYVYQGL
20 μg NLLRLTGWFFRKSIINFEKLFFRKRGYVYQGL	410 ± 49	0.3 ± 0.4	250 ± 11
10 μg NLLRLTGWFFRKSIINFEKLFFRKRGYVYQGL	360 ± 13	0.3 ± 0.4	100 ± 10
5 μg NLLRLTGWFFRKSIINFEKLFFRKRGYVYQGL	130 ±3.3	0 ± 0	35 ± 6.6
20 μg NLLRLTGWFFRKRGYVYQGLFFRKSIINFEKL	150 ± 6	0 ± 0	380 ± 12
10 μg NLLRLTGWFFRKRGYVYQGLFFRKSIINFEKL	30 ± 3	0 ± 0	83 ± 5

In this and other experiments, the epitope proximal to the heat shock protein binding domain exhibited the strongest immune response, and thus the positioning of the selected epitopes selected for the vaccine formulations of the invention may be positioned to contribute maximally to the overall immunogenicity of the formulation, whether administered in the absence of heat shock proteins or administered as complexes with heat shock proteins.

EXAMPLE 22

In the following experiments, admixtures of tandem hybrid antigens were evaluated for immunogenicity. In addition to the H2-K^b Class I peptides from ovalbumin (SIINFEKL) and from VSV (RGYVYQGL), the H2-K^b β-casein peptide IAYFYPEL and the Sendai virus peptide FAPGNYPAL were also used. In another experiment, two tandem hybrid antigens with the same antigenic peptides in alternate configurations were admixed. Strong immune responses to four epitopes were elicited.

All of the formulations herein included 1 mM ADP. In one experiment described below, ADP was omitted.

(200-72-04)

Immunogen	Number of Spots per 300,000 cells			
	SIINFEKL	RGYVYQGL	IAYFYPEL	FAPGNYPAL
9.6 µg NLLRLTGWFFRKSIINFEKLFFRKRGYVYQGL	537 ± 16	150 ± 10	4.7 ± 0.8	5.7 ± 2.5
9.7 µg NLLRLTGWFFRKIAYFYPELFFRKFAPGNYPAL	1.7 ±1.1	1.7 ± 0.8	128 ± 9.2	136 ± 6.6
9.6 µg NLLRLTGWFFRKSIINFEKLFFRKRGYVYQGL plus 9.7 µg NLLRLTGWFFRKIAYFYPELFFRKFAPGNYPAL	363 ± 31	256 ± 5.3	127 ± 7.9	155 ± 28

5 S200-72-13

Immunogen	Number of Spots per 300,000 cells			
	SIINFEKL	RGYVYQGL	IAYFYPEL	FAPGNYPAL
9.6 µg NLLRLTGWFFRKIAYFYPELFFRKFAPGNYPAL plus 9.6 µg NLLRLTGWFFRKSIINFEKLFFRKRGYVYQGL	388 ± 6.8	72 ± 5.0	402 ± 17	379 ± 30
9.6 µg NLLRLTGWFFRKRGYVYQGLFFRKSIINFEKL Plus 9.6 µg NLLRLTGWFFRKIAYFYPELFFRKFAPGNYPAL	76 ± 1.9	159 ± 8.3	115 ± 20	172 ± 5.9

S200-72-13

Immunogen	Number of Spots per 300,000 cells			
	SIINFEKL	RGYVYQGL	IAYFYPEL	Medium
9.6 µg NLLRLTGWFFRKSIINFEKLFFRKRGYVYQGL	450 ± 10	273 ± 12	3.0 ± 1.4	0.33 ± 0.41
9.6 µg NLLRLTGWFFRKRGYVYQGLFFRKSIINFEKL	82 ± 4	445 ± 30	1.3 ± 0.41	0 ± 0
9.6 µg NLLRLTGWFFRKSIINFEKLFFRKRGYVYQGL plus 9.6 µg NLLRLTGWFFRKRGYVYQGLFFRKSIINFEKL	202 ± 7.6	188 ± 24	1.0 ± 0.7	0.67 ± 0.41

S200-72-13, no ADP

Immunogen	Number of Spots per 300,000 cells			
	SIINFEKL	RGYVYQGL	IAYFYPEL	Medium
9.6 µg NLLRLTGWFFRKSIINFEKLFFRKRGYVYQGL	228 ± 2.5	126 ± 2.9	1.7 ± 0.4	0 ± 0
9.6 µg NLLRLTGWFFRKRGYVYQGLFFRKSIINFEKL	83 ± 9	189 ± 19	13 ± 15	0.33 ± 0.41
9.6 µg NLLRLTGWFFRKSIINFEKLFFRKRGYVYQGL plus 9.6 µg NLLRLTGWFFRKRGYVYQGLFFRKSIINFEKL	115 ± 7.8	86 ± 11	0.33 ± 0.41	0 ± 0

5

EXAMPLE 23

In the following experiment, up to five antigenic peptides are delivered and induce immunogenicity without co-administered HSP70, when administered as an admixture of two tandem hybrid antigens and a single hybrid antigen to B6 mice. The tandem hybrid antigens included VSV and ovalbumin peptides in one, and β-casein and Sendai virus peptides in the other. The single hybrid antigen contained NS2-114 influenza peptide (RTFSFQLI).

S200-72-15

Immunogen	Number of Spots per 300,000 cells				
	SIINFEKL	RGYVYQGL	IAYFYPEL	FAPGNYPAL	RTFSFQLI
9.6 μg NLLRLTGWFFRKRGYVYQGL FFRKSIINFEKL plus 19 μg NLLRLTGWFFRKIAYFYPELF FRKFAPGNYPAL	67 ± 6.1	205± 20	229 ± 28	266 ± 33	0 ± 0
9.6 μg NLLRLTGWFFRKRGYVYQGL FFRKSIINFEKL plus 19 μg NLLRLTGWFFRKIAYFYPELF FRKFAPGNYPAL plus 12.2 μg NLLRLTGWFFRKRTFSFQLI	156 ± 3.3	299 ± 18	175 ± 12	125 ± 3.3	33 ± 4.7

EXAMPLE 24

The immunogenicity of the foregoing single hybrid antigens administered without heat shock protein were evaluated in combination with helper T cell epitopes present in a hybrid antigen. In most experiments, a H2-K^b Class II epitope from ovalbumin, amino acids 323-339, TEWTSSNVMEERKIKV, was used (i.e., the hybrid antigen had a sequence of

NLLRLTGWFFRKTEWTSSNVMEERKIKV). Inclusion of the Class II peptide-containing hybrid antigen increased the response to the Class I epitope on the average of about seven fold.

(250-72-08)

Class I hybrid peptide-containing immunogen	Number of Spots per 300,000 cells			
	Response to Class I epitope when Class I hybrid antigen administered	Response to Class I epitope when Class I and Class II hybrid antigen admixture is administered	Medium	Splenocytes
24.2 µg NLLRLTGWFFRKDAPIYTNV	2 ± 1.9	13 ± 3.9	0.7 ± 0.4	0 ± 0
24.9 µg NLLRLTGWFFRKSSWDFITV	18 ± 0.7	98 ± 5.8	0.7 ± 0.8	0.7 ± 0.4
25.4 µg NLLRLTGWFFRKRTFSFQLI	5.3 ± 1.5	43 ± 7.6	0.3 ± 0.4	0 ± 0
25.5 µg NLLRLTGWFFRKIAYFYPEL	11 ± 3	73 ± 9.8	0 ± 0	0 ± 0

EXAMPLE 25

The effect on immunogenicity of hybrid antigens co-administered with various hybrid antigens containing H2-Kb Class II peptides, in the absence of heat shock protein, were evaluated. The Class I peptides were either SSWDFITV or DAPIYTNV; Class II peptides included the ovalbumin peptide mentioned above, a Class II peptide from tetanus toxoid NNFTVSFWLRVPKVSASHL (i.e., the hybrid antigen has a sequence of NLLRLTGWFFRKNNFTVSFWLRVPKVSASHL), or a HBVc (amino acids 128-140) peptide, TPPAYRPPNAPIL.

Immunogen	Number of Spots per 300,000 cells	
	Medium	SSWDFITV
24.9 μg NLLRLTGWFFRKSSWDFITV	3.0 ± 0.7	78 ± 3.9
24.9 μg NLLRLTGWFFRKSSWDFITV plus 27.4 μg NLLRLTGWFFRKTPPAYRPPNAPIL	8.0 ± 3.1	84 ± 7.1
24.9 μg NLLRLTGWFFRKSSWDFITV plus 33.6 μg NNFTVSFWLRVPKVSASHLGSGNLLRLTGW	3.7 ± 1.1	315 ± 15
24.9 μg NLLRLTGWFFRKSSWDFITV plus 36.4 μg HWDFAWPWNGSGNNFTVSFWLRVPKVSASHL	2.7 ± 2.0	135 ± 5.7
24.9 μg NLLRLTGWFFRKSSWDFITV plus 34.7 μg NLLRLTGWFFRKTEWTSSNVMEERKIKV	1.7 ± 0.4	229 ± 12

Thus, a helper T cell epitope may be included in a hybrid antigen as the only epitope, and administered as an admixture with other hybrid antigens containing Class I epitope(s), or the helper T cell epitope can be included in a tandem hybrid antigen as one of the epitopes. These are merely exemplary of the numerous variations upon the hybrid antigen compositions of the invention.

EXAMPLE 26

In a similar fashion to the previous example, the immunogenicity of a tandem hybrid antigen was evaluated with and without co-administration of a hybrid antigen containing the ovalbumin Class II peptide.

S250-72-12

Immunogen	Number of Spots per 300,000 cells		
	IAYFYPEL	FAPGNYPAL	Medium
19 µg NLLRLTGWFFRKIAYFYPELFFRKFAPGNYPAL	9.3 ± 4.7	17 ± 9	0.7 ± 0.6
19 µg NLLRLTGWFFRKIAYFYPELFFRKFAPGNYPAL Plus 20.8 µg NLLRLTGWFFRKTEWTSSNVMEERKIKV	44 ± 5.1	58 ± 5.2	0.7 ± 0.6

250-72-15

Immunogen	Number of Spots per 300,000 cells IAYFYPEL
25.5 µg NLLRLTGWFFRKIAYFYPEL	3.7 ± 3.1
25.5 µg NLLRLTGWFFRKIAYFYPEL plus 34.7 µg NLLRLTGWFFRKTEWTSSNVMEERKIKV	133 ± 11
25.5 µg NLLRLTGWFFRKIAYFYPEL plus 25 Mg NLLRLTGWFFRKSIINFEKL	88 ± 9.9

EXAMPLE 27

Similar experiments with hybrid antigens comprising a helper T cell epitope co-administered with at least one tandem hybrid antigen, in the absence of co-administration of a heat shock protein, were also carried out.

250-72-12

Immunogen	Number of Spots per 300,000 cells				
	Medium	SIINFEKL	RGYVYQGL	IAYFYPEL	FAPGNYPAL
24 µg NLLRLTGWFFRKIAYFYPELF FRKFAPGNYPAL	0.7 ± 0.6	NT	NT	9.3 ± 4.7	17 ± 8.7
24 µg NLLRLTGWFFRKIAYFYPELF FRKFAPGNYPAL plus 21 µg NLLRLTGWFFRKTEWTSSNV MEERKIKV	0.7 ± 0.6	NT	NT	44 ± 5.1	67 ± 5.5
15 µg NLLRLTGWFFRKFAPGNYPA L	0 ± 0	NT	NT	0.3 ± 0.6	4.3 ± 3.2
15 µg NLLRLTGWFFRKFAPGNYPA L plus 21 µg NLLRLTGWFFRKTEWTSSNV MEERKIKV	0 ± 0	NT	NT	2.3 ± 2.1	58 ± 5.2

EXAMPLE 28

An immunization study using hybrid antigens containing human Class I (HLA-A2) epitopes was performed in HHD II mice as described above. Animals were immunized with a complex made from 5 μg hsp70 and 33 μg NLLRLTGWFFRKYMDGTMSQV. The ELISPOT results in cells per 300,000 were: Medium, 1.33 ± 0.58; splenocytes 1 ± 0; splenocytes plus YMDGTMSQV 123 ± 13; and splenocytes plus IMDQVPFSV 4 ± 1.

EXAMPLE 29

In another experiment using HHDII mice, an immunogenic HLA-A2 epitope from Trp-2 was used (SVYDFFVWL). Because this epitope is also a H2-Kb epitope, and the HHDII mice are on a B6 mouse (H2-Kb) background, an immune response induced against the Trp-2 peptide represents a breaking of tolerance to a self-epitope in the mouse model. The results of this experiment demonstrated that tolerance to this self-epitope was broken, and the present invention is further directed to methods of breaking tolerance by administering the hybrid antigens and complexes of the invention.

HHDII-200-72-03

Immunogen	Number of Spots per 300,000 cells			
	Medium	SVYDFFVWL	IMDQVPFSV	YMDGTMSQV
4.33 µg NLLRLTGWFFRKSVYDFFVWL plus 25 µg hsp70	0.5 ± 0.71	166 ± 25	2.0 ± 1.4	3.5 ± 0.71
8.66 µg NLLRLTGWFFRKSVYDFFVWL plus 25 µg hsp70	3.5 ± 0.71	114 ± 11	7.7 ± 2.1	11 ± 3.1
4.1 µg NLLRLTGWFFRKYMDGTMSQV plus 25 µg hsp70	3.0	1.0	2.0 ± 1.4	74 ±2.8
4.1 µg NLLRLTGWFFRKIMDQVPQV plus 25 µg hsp70	1.0 ± 1.4	2.0 ± 2.0	984 ± 26	2.3 ± 1.5

EXAMPLE 30

HHDII mice were used to evaluate the immunogenicity of complexes of hsp70 and three hybrid antigens comprising certain of the HIV viral component epitopes set forth in Example 27.

Immunogen	Number of Spots per 300,000 cells			
	Medium	ILKEPVHGV	VIYQYMDDL	SLYNTVATL
36 µg NLLRLTGWFFR KILKEPVHGV + 25 µg hsp70	1.0 ± 1.0	34 ± 12	0 ± 0	NT
36 µg NLLRLTGWFFR KVIYQYMDDL + 25 µg hsp70	0 ± 0	0.67 ± 0.58	24 ± 6.1	NT
36 µg NLLRLTGWFFR KSLYNTVATL + 25 µg hsp70	0.67 ± 0.58	NT	NT	140 ± 6.7

NT=not tested

EXAMPLE 31

Admixtures of hybrid antigens containing H2-Kb epitopes complexed with hsp70 were evaluated for immunogenicity in B6 mice as described above.

OBS-72-01

Immunogen	Number of Spots per 300,000 cells				
	Medium	Splenocytes	SIINFEKL	FAPGNYPAL	IAYFYPEL
2 μg NLLRLTGWFFRKSIINFEKL + 13.7 μg hsp70	1	2 ± 1	148 ± 11	7 ± 2	3 ± 2
10 μg NLLRLTGWFFRKIAYFYPEL + 13.7 μg hsp70	0	2 ± 2	3 ± 1	8 ± 2	47 ± 13
10 μg NLLRLTGWFFRKFAPGNYPAL + 13.7 μg hsp70	3	3 ± 3	3 ± 2	83 ± 6	6 ± 1
2 μg NLLRLTGWFFRKSIINFEKL + 10 μg NLLRLTGWFFRKIAYFYPEL + 27.4 μg hsp70	2	4 ± 2	94 ± 4	9 ± 3	29 ± 4
2 μg NLLRLTGWFFRKSIINFEKL + 10 μg NLLRLTGWFFRKFAPGNYPAL + 27.4 μg hsp70	3	3 ± 0	169 ± 7	157 ± 27	4 ± 2
10 μg NLLRLTGWFFRKIAYFYPEL + 10 μg NLLRLTGWFFRKFAPGNYPAL + 27.4 μg hsp70	3	3 ± 3	4 ± 3	46 ± 8	39 ± 2
2 μg NLLRLTGWFFRKSIINFEKL + 10 μg NLLRLTGWFFRKIAYFYPEL + 10 μg NLLRLTGWFFRKFAPGNYPAL + 41 μg hsp70	1	5 ± 2	149 ± 19	61 ± 5	60 ± 7

EXAMPLE 32

The immunogenicity of tandem hybrid antigens complexed with hsp70 was studied in B6 mice.

S200-72-01

Immunogen	Number of Spots per 300,000 cells			
	Medium control	Unpulsed control	SIINFEKL	RGYVYQGL
5.6 µg hsp70 + 3 µg NLLRLTGWFFRKSIINFEKL	0.33 ± 0.41	0.67 ± 0.41	43 ± 9.2	1 ± 0
11.2 µg hsp70 + 5.9 µg NLLRLTGWFFRKRGYVYQGL	0.33 ± 0.41	0.33 ± 0.41	1 ± 0.71	102 ± 16
11.2 µg hsp70 + 3 µg NLLRLTGWFFRKSIINFEKL + 5.9 µg NLLRLTGWFFRKRGYVYQGL	0.67 ± 0.82	1.7 ± 0.41	182 ± 11	113 ± 10
5.6 µg hsp70 + 4.8 µg NLLRLTGWFFRKSIINFEKLFFRKRGYVYQGL	0 ± 0	4 ± 1.4	456 ± 19	113 ± 1.1
11.2 µg hsp70 + 9.6 µg NLLRLTGWFFRKSIINFEKLFFRKRGYVYQGL	0 ± 0	10 ± 3.3	505 ± 57	90 ± 11
22.4 µg hsp70 + 19.2 µg NLLRLTGWFFRKSIINFEKLFFRKRGYVYQGL	0.67 ± 0.82	1.7 ± 0.41	289 ± 26	130 ± 12
5.6 µg hsp70 + 4.8 µg NLLRLTGWFFRKRGYVYQGLFFRKSIINFEKL	0.33 ± 0.41	2.3 ± 0.41	72 ± 9.5	98 ± 9.2
11.2 µg hsp70 + 9.6 µg NLLRLTGWFFRKRGYVYQGLFFRKSIINFEKL	2 ± 0	2.3 ± 1.5	370 ± 16	617 ± 23
22.4 µg hsp70 + 19.3 µg NLLRLTGWFFRKRGYVYQGLFFRKSIINFEKL	0.67 ± 0.41	4.0 ± 2.1	336 ± 7.8	728 ± 12

5

The present invention is not to be limited in scope by the specific embodiments describe herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the contents of which are incorporated herein by reference in their entireties.